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Applications of a liquid membrane in Taylor flow regime (LMTF) on separation processes and design of fermentation hybrid systems

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Dedication

To my family. They always have supported me, believed in me, and encouraged me to achieve my goals.

To my advisors. They always are teaching me, not only on science but about life as well.

To my friends. They never let me down and spent with me nice moments during this journey.

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Abstract

In this book, it is shown a proof of concept and the performance assessment of a novel liquid membrane in Taylor flow applied to the lactic acid (LA) removal. Liquid-liquid equilibria (LLE) of potential membrane phases for LA removal were experimentally measured and a LLE model is proposed to fit the values of the of the distribution coefficient and chemical equilibrium constants for this kind of systems. Additionally, molecular toxicity tests of the potential liquid membranes on the lactic acid bacteria *Lactobacillus casei* ATCC 393 were carried out in order to have a membrane phase for LA removal with a good compromise between a high value of the chemical equilibrium constant and a relatively low molecular toxicity.

The performance of the liquid membrane in Taylor flow (LMTF) in terms of hydrodynamics and mass transfer was tested for LA removal. The LA removal is favored at low injection times and high droplet velocities by providing the suitable space-time to achieve the mass transfer at the operating condition used. A semi-empirical model for calculation of the overall volumetric mass transfer coefficients (OVMTC) was developed and their empirical parameters were fitting by using the experimental results.

The LMTF was integrated with a batch lactic acid fermentation in order to remove the LA during fermentation. This hybrid process was experimentally assessed in terms of LA productivity and yields comparing it with a conventional batch fermentation. The LA produced increases by 41%, the glucose consumption increases by 68% and the biomass production increases by 12%. The glucose consumption is higher than LA and biomass production, which is in agreement with the effect of the membrane phase on *Lactobacillus casei* ATCC 393 which promotes the glucose consumption instead of biomass and LA production. The model for the hybrid process was developed using material balances for the fermenter and the model of the OVMTC of the LMTF for LA removal. The model shows some slight differences as compared with experimental results because the model does not take into account the toxicity effects of the membrane phase on the lactic acid bacteria. In the model is included a LMTF system with multi-channels. The effect of the number

of channels of the LMTF is modeled and its impact on productivity, fermentation time, and final biomass concentration are analyzed.

From the experimental results, it can say that the LMTF is a promising technology for removal of LA, from both aqueous lactic acid solutions and fermentation broths. The LMTF can be integrated with fermentation processes to remove metabolites and enhance both LA and biomass productivity, however, molecular toxicity issues could reduce LA to glucose yield.

Within this book, every section of each chapter is self-contain and can be read independently.

***Keywords*:** Process Intensification, hybrid system, liquid membrane in Taylor flow, lactic acid fermentation, liquid-liquid equilibria, molecular toxicity.

Aplicaciones de una membrana líquida en flujo de Taylor sobre procesos de separación y diseño de sistemas híbridos de fermentación.

Resumen

En éste libro se presentan la prueba de concepto y la evaluación de desempeño de una nueva membrana líquida en flujo de Taylor aplicada a la remoción de ácido láctico (AL). Experimentalmente se midieron los equilibrios líquido-líquido (ELL) de fases membranas potenciales para la remoción de AL y se propuso un modelo de ELL para ajustar los valores de coeficiente de distribución y de la constante de equilibrio químico para este tipo de sistemas. Adicionalmente, se realizó la evaluación de toxicidad molecular de las membranas liquidas potenciales sobre la bacteria ácido láctica *Lactobacillus casei* ATCC 393 con el fin de obtener una fase membrana para la remoción de AL con un alto valor de la constante de equilibrio químico y una baja toxicidad molecular.

Se evaluó el desempeño de la membrana líquida en flujo de Taylor (MLFT) en términos hidrodinámicos y la transferencia de masa para la remoción de AL. Se observó que la remoción de AL es favorecido a bajos volúmenes de inyección y altas velocidades de las gotas siempre y cuando se proporcione un tiempo espacial suficiente para alcanzar la trasferencia de masa a la condición de operación usada. Se desarrolló también un modelo semi-empírico para calcular los coeficientes globales volumétricos de trasferencia de masa (CGVTM), en el cual se ajustan los parámetros empíricos usando los resultados experimentales.

El sistema de MLFT se integró con una fermentación ácido láctica en batch con el fin de remover el AL durante la fermentación. El sistema híbrido antes mencionado, fue evaluado experimentalmente en términos de la productividad y rendimientos, comparándolos con los de una fermentación convencional en batch. Se observó que incrementaron el AL producido en un 41%, el consumo de glucosa un 68% y la producción de biomasa un 12%. El consumo de glucosa es mayor que el AL y la biomasa producidos, lo que está en concordancia con el efecto de la fase membrana sobre la bacteria ácido láctica *Lactobacillus casei* ATCC 393, el cual promueve el consumo de glucosa en lugar de la producción de AL. Se desarrolló un modelo para el sistema híbrido usando los balances de materia para el bioreactor y el modelo de CGVTM de la MLFT para la remoción de AL. Se observan algunas diferencias entre los valores predichos por el modelo y los obtenidos experimentalmente debido a que el modelo no tiene en cuenta loes efectos tóxicos de la fase membrana sobre la bacteria ácido láctica usada. El modelo también incluye un sistema multicanal

para la MLFT. Se modela el efecto del número de canales de la MLFT y se analiza su impacto en la productividad, tiempo de fermentación y concentración final de biomasa.

A partir de los resultados experimentales, se puede decir que la MLFT es una tecnología prometedora para la remoción de AL, tanto de una solución acuosa como de la caldos de fermentado. El sistema de MLFT puede ser integrado con un proceso de fermentación para remover los metabolitos y mejorar la productividad tanto de AL como de biomasa, sin embargo, los efectos de toxicidad molecular podrían reducir el rendimiento de AL a glucosa.

Cada sección de capítulo dentro de éste libro esta auto-contenida y puede leerse independientemente.

Palabras clave: Intensificación de procesos, sistema híbrido, membrana líquida en flujo de Taylor, fermentación ácido láctica, equilibrio líquido-líquido, toxicidad molecular.

Table of contents

1. Chapter 1: Introduction

Liquid membrane (LM) technology is a separation process with great potential for industrial applications due to modular design, easy to scale-up, selectivity, low energy requirements, and a remarkable trend for low environmental impact [1,2], which have attracted the attention of scientist and engineers due to its advantages over solid membranes and liquid-liquid extraction [3]. However, the applications for LMs are limited due to stability issues [2].

The liquid membrane in Taylor flow regime (LMTF) is a new kind of contact for LMs, which promises overcome the stability problems keeping the high flux of conventional LMs. A challenge in understanding this recently developed membrane technology is that currently is not known the effect of the operating conditions on the performance of the LMTF for solute removal. Knowing the main variables of the LMTF and how they are related to operating conditions allows designing a suitable LMTF process for specific applications.

The LMTF as the conventional LMs is an advanced technique for recovery, purification, and abatement of substances that can be integrated to other separation or reactive fermentative-processes [3] to increase the performance and the productivity applying process intensification philosophy [4].

Production of specific substances by fermentation such as alcohols, organic acids, and pharmaceutical compounds, among others, are inefficient processes because they require several separation and purification steps which generally have a high cost [5,6], the freshwater consumption is high [5] and the final concentration of the main metabolite of fermentation (product) is limited by end-product inhibition [5–7]. Through an integrated fermentation-separation process (extractive fermentation process), it is possible to remove the fermentation product during the fermentation to improve the productivity of the fermentation process reducing the end-product inhibition [8]. Several researches have integrated fermentative processes for production of specific metabolites, such as, ethanol [9], butanol [10], L (+) glutamic acid [11] and succinic acid [12], with specific separation

technologies as pervaporation, micro and nano-filtration, electrodialysis and Donnan dialysis, gas stripping and liquid-liquid extraction, among others.

The LMTF is a potential separation technology to be integrated into a fermentation process to increase productivity and reduce the end-product inhibition, which currently has not been tested with any fermentative process.

The production of lactic acid (LA) by fermentation, which corresponds to 90% of the total production, has around 100 years without significant technological changes [13–15]. The corresponding costs of separation and final purification of LA is around 50% of the total cost of the process [13,16–18]. This fermentative process is interesting for its integration to LMTF for in-situ removal of LA and, in this way, reducing their known drawbacks.

This thesis has two main aims: First, to assess the effect of the operating conditions of the LMTF on its performance for solute removal and understand its behavior from a hydrodynamic and a mass transfer points of view. Second, to integrate the LMTF to a fermentative process as a new alternative to overcome the common drawbacks of conventional fermentative processes.

Below, it is shown a brief description of the conventional liquid membrane processes, their main transport mechanisms, and the common drawbacks. A general description of the liquid membrane in Taylor flow is also presented. Subsequently, it is shown an overview of several separation technologies used in hybrid systems with fermentative processes. This chapter includes a brief description of the thesis outline.

1.1 Liquid membranes and the liquid membrane in Taylor flow

A liquid membrane is a liquid semi-permeable barrier which spatially separates two fluid phases, donor phase and receiving phase [2,3]. Donor phase (*D*), contains the solute which will be transported through the membrane phase (*M*) to the receiving phase (*R*) as is shown in Figure 1. Generally, the membrane phase is composed of organic substances while the donor and receiving phases are aqueous solutions [19].

The liquid membrane technology is a perstraction process (which involves extraction and back-extraction processes with membrane separation in a single stage) used for separation or concentration of substances [2,19]. LM technology has been applied in several fields such as hydrometallurgy, biotechnology, medical and in general for the treatment of industrial wastewaters [1–3]. LM has

been used for removal of toxic metal from effluents, recovery of the metabolites produced on fermentations, removal of toxic gas agents and, removal of organic or inorganic compounds from industrial effluents, among others [2,3].

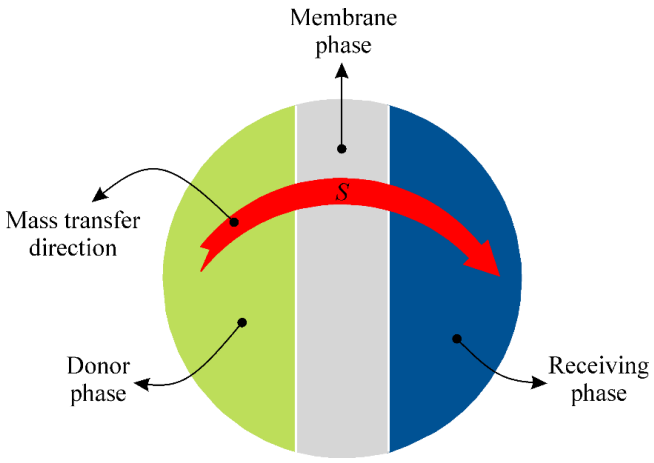


Figure 1. Scheme of the liquid membrane and the involved phases for solute (*S*) removal.

LM technology, unlike the conventional liquid-liquid extraction, requires a low amount of solvent due to it is continuously in-situ regenerated, is less energy consuming, the number of stages is reduced, and is not thermodynamically limited because of the continuous solute removal from the membrane phase by the receiving phase [2,3,19]. Therefore, LM technology has great potential to be applied in process intensification [2,20–31]. LMs can reduce the operating costs, environmental impact, and energy consumption when they are combined with other separation or reactive processes, such as bioremediation, selective oxidation, fermentation, distillation, absorption, and adsorption [2–4,26,32–36].

There are three types of liquid membranes: bulk, supported and emulsion liquid membranes. Bulk liquid membranes have been used to test both mass transport and kinetics at lab-scale due to this LM is limited by its low specific interface area [3]. Supported liquid membranes (SLM) and emulsion liquid membranes (ELM) have potential on applications in industrial scale because they provide large interfacial area among the phases, extraction, and stripping are in one stage, simple operation and it is possible to process high quantities of compounds (from donor phase) using small volumes of the membrane phase [2,24]. ELM offers higher mass transport rates than their counterpart SLM which requires regeneration steps due to membrane liquid phase losses during the process operation [2]. ELM requires mixing steps for the formation of stable emulsions that later must be disrupted by thermal breakdown or electrical demulsification and separated in decanters in subsequent steps

[3,19,37]. In order to stabilize the emulsions, usually, it is necessary to add surfactants or surface-active substances [2,3,19] that also hinders the decantation processes.

In the LM process, there are two main transport mechanisms: passive diffusion (or simple diffusion) and facilitated transport [25]. Facilitated transport occurs faster than passive diffusion and increases monotonically as the driving-force, given by the difference of the chemical potential between the donor and membrane phases, increases [25]. In facilitated transport, an active agent or carrier is added to the membrane phase [2,25], and it reacts with the solute in the interface *D*/*M* in order to produce a complex. This complex is transported from the interface *D*/*M* to the interface *M*/*R*, and here, the complex releases the solute on the receiving phase. The use of a carrier provides selectivity for a specific solute and high mass transport rate of the solute through the membrane phase [38].

Currently, a new type of contact among the phases of the LM process has been proposed [39] and developed as perstraction process with the potential to overcome the abovementioned drawbacks of the LMs. This type of LM keeps a high mass transfer of solute among the phases without using surfactants and reducing the number of steps of the ELM. This LM uses Taylor flow regime extending it to three-phases as the way of contact of the phases of the LM (donor, membrane and receiving phases) and it takes advantage of the enhanced mass transfer of this flow regime [40]. The mass transfer and the particular hydrodynamics of Taylor flow have been applied in mixing, separation and reactive processes in order to improve the performance of these processes [41–47]. The liquid membrane process was named liquid membrane in Taylor flow regime (LMTF), and as a proof concept, it was tested for lactic acid removal [39]. While the three phases of the LMTF are flowing in the same direction within a channel, the transport of the solute through the LMTF (Figure 2) is carried out from the donor phase to the membrane phase and from here to the receiving phase. The donor and receiving phases are aqueous droplets within the tube (or channel) while the membrane phase is the continuous phase or liquid slugs in the Taylor flow. Using this configuration, all phases of the LMTF are in motion and interfaces are continuously renewed.

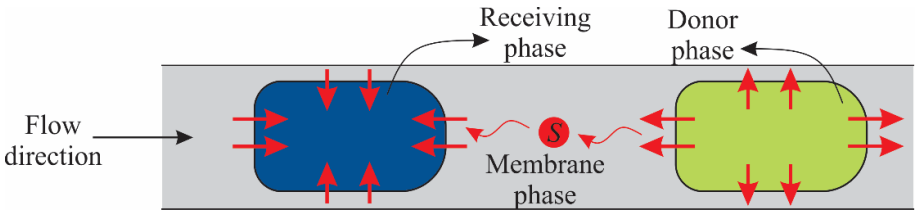


Figure 2. Solute removal (*S*) through the liquid membrane in Taylor flow.

In chapter 2 (Figure 3) of this book, experimental liquid-liquid equilibria (LLE) is shown of potential membranes phases (organic phases) with aqueous LA solutions. Thus, the experimental extraction capacity for potential organic phases for LA removal is measured. Additionally, it is presented the main mechanisms of reaction between LA and the carrier of the tested potential membrane phases. Also, a mathematical model is proposed and developed that predicts the corresponding LLE.

In chapter 3 (Figure 3), the potential membrane phases for LA removal were tested in order to achieve a proper membrane phase for LA removal from a fermentation broth by *Lactobacillus casei* ATCC 393, based on their molecular toxicity on the lactic acid bacteria (LAB). Usually, the membrane phases or the organic phases of the reactive extraction with high LA removal capacity are also toxic for the microorganism [48]. Therefore, the molecular toxicity of each pure substance that composes the potential membrane phase was tested on the abovementioned LAB elucidating the main physicochemical properties that produce a high toxicity on the bacteria based on cell growth, LA production, and glucose consumption. Also, molecular toxicity and LLE were measured for mixtures of the less toxic carrier with several proportions of a non-toxic with a medium toxic diluent.

In chapter 4 (figure 3), an experimental set-up for testing the LMTF is presented. Several operating conditions of the LMTF for LA removal are experimentally tested to characterize the main variables of the LMTF that affects both its LA removal performance and its hydrodynamic behavior. Also, in chapter 4, the overall volumetric mass transfer coefficients (OVMTC) involved on the LMTF are calculated from the experimental results using three empirical models, one of which was obtained in this work based on dimensional analysis.

1.2 Integration of the liquid membrane in Taylor flow with a fermentation process

Currently, fermentation processes have several drawbacks which make it an inefficient process. They consume high quantities of fresh water for each batch [5], the product is highly diluted in the fermentation broth, and inhibitory compounds are produced during fermentation [5–7]. Generally, the main product of the fermentation inhibits the cell growth. Therefore, the final concentration of the product is limited [5–7]. Additionally, the subsequent steps for separation and purification require several units, each one with yield losses and several of them are high energy demanding [5,6].

Several technologies to improve the fermentation processes have been proposed such as feed-batch reactor operation, multi-phase reactor operation and bio-catalysis. Bio-catalysis has been the most used. However, it presents losses of cell viability and enzyme activity [5]. In-situ product removal (ISPR) has great potential to overcome the drawbacks mentioned above for fermentation processes. ISPR involves product removal during the fermentative process and can be applied internally or externally to the fermenter. ISPR has been applied to fuel, chemical, pharmaceutical, and food products [5]. From the point of view of the microorganisms, ISPR operation can be carried out with direct or indirect contact in an external or internal unit [5].

These hybrid processes or units are focused on increasing the fermentation product concentration, removing the inhibitor compounds and reducing the number of steps in the global process allowing continuous operation. In a hybrid fermentation-separation system, the product removal and the reaction step are possible to carry out in a recycle loop. Moreover, the product recovery can be achieved in the same vessel and, sometimes, byproducts may need to be isolated [5].

Several separation processes have been used to achieve a hybrid process where the product is continuously removed from fermentation. Liquid-liquid extraction, adsorption, and membrane technologies are the most used in the development of hybrid processes [6,49–52]. The use of one or another separation technology is highly related to the substance of interest to remove from the fermentation broth.

Filtration process (micro-filtration) was used to remove cells and to recover the fermentation product. It was used in the lactic acid fermentation where both fouling and substrate loss were observed. Thus, it was necessary the treatment of the membranes [51].

The adsorption process requires the use of ion exchange resins and usually two-bed columns. While one column is removing the product, the second one is in the regeneration step. This technology has been used for carboxylic acid removal. It provides high product concentration, avoids the use of pH control and reduces inhibition by a fermentation product [53].

Electrodialysis is a membrane technology that requires to form a carboxylate from the fermentation broth and electro-conversion of carboxylate into carboxylic acid. Lactic acid fermentation has been one of the most studied processes using this technology [51,54]. The pH must remain slightly higher but close to a value of 6. It is known, that the lactic acid in an undissociated form reduces the microbial activity and at low pH (pH<5) the formation of the undissociated form of lactic acid is

promoted [51]. This hybrid process, as adsorptive fermentation, provides pH control on the lactic acid production reducing product inhibition and providing higher fermentation yields than conventional batch fermentations [7,54]. An intensified electrodialysis process, LA recovery in electro-enhanced dialysis (REED) were developed and tested as a promising alternative to be integrated into a fermentation process [55].

Liquid-liquid extraction integrated to fermentation has been widely studied and usually, it is called hybrid liquid-liquid extraction-fermentation [6] or extractive fermentation [6,56]. In this process, the extraction solvent must be highly efficient and, in general, must accomplish the following conditions [6,50]:

a) Low or non-toxic on the microorganism of the fermentation.
b) Show a high distribution coefficient.
c) High selectivity for the solute.
d) Low solubility in the aqueous phase.
e) High-density difference with the fermentation broth to ensure two-phase separation.
f) Low viscosity and high interfacial tension with low trend to emulsify in the fermentation broth.
g) High stability.
h) Low cost.

Removal of organic acids from the fermentation broth by reactive liquid extraction has been the main application for this separation process [18,57–59], where LA removal has been studied [60–63]. The solvent recovery step or co-extraction step increases the costs due to the use of a co-solvent. Due to this and the toxicity effects of some solvents, a modified liquid extraction using indirect contact has been used. It was achieved using a porous non-wetted membrane between the two phases. This process is called for some authors as perstraction [8,9]. The membrane is used as a contactor to provide a high interfacial area between the phases and to reduce the toxicity of the solvent on the microorganism.

LMs are another membrane technology with the potential to be integrated into a fermentative process. There are several studies on the integration of LMs in fermentative processes, especially for organic acid removal [48,64–68]. For LA removal both SLM and ELM have been used and tested, where the recent trend is on the use of ionic liquids within the membrane phase [29,69–77]. Also, some studies of LM on in-situ LA removal have been published, showing it as promising methods for efficient LA production [48,58].

In this thesis, the LMTF is tested for in-situ LA removal during LA fermentation by *Lactobacillus casei* ATCC 393. In chapter 5 (Figure 3), the experimental set-up of the LMTF is integrated to a batch LA fermentation of 50 mL (hybrid system), and the effects of the LA removal during fermentation through the LMTF on cell growth, LA production, and glucose consumption are evaluated. As compared to a conventional batch LA fermentation, the experimental results show that it is possible to increase productivity, biomass concentration and LA produced using this LMTF hybrid system. Additionally, a model for the hybrid system is developed from the experimental results taking into account LA kinetics and OVMTC of the LMTF. The effect of LMTF system with several channels on the LA fermentation is simulated, assessing parameters such as, productivity, final biomass concentration within the fermenter, total LA mass achieved, and the pH within the fermenter. The results show that the LMTF is also an alternative for pH control of LA fermentative processes.

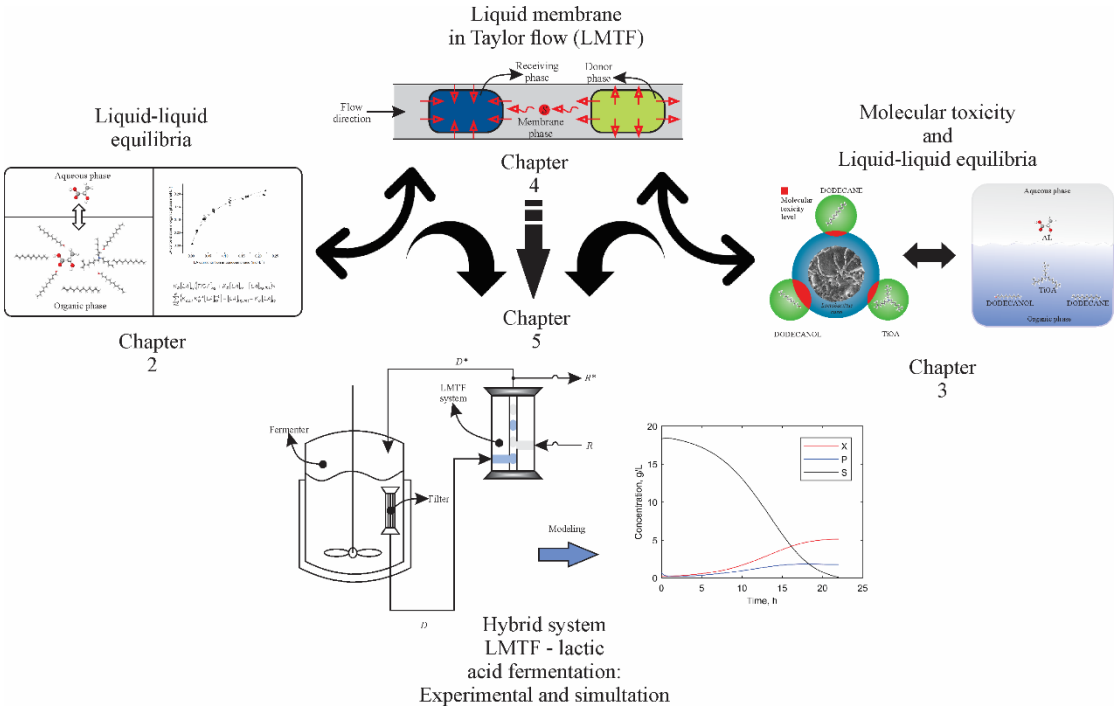


Figure 3. Schematic overview of the chapters through this book.

Chapters 2 – 4 of this book are the product of the design of a hybrid system where the LMTF is integrated to a fermentative process for *in situ* product removal (chapter 5). Each chapter of this book is an important stage that has to be carried out to successfully achieve the integration of the LMTF to a fermentative process. Hence, chapters 2 – 4 are related in between and conducts to chapter 5

(Figure 3). To achieve this aim, the LA fermentation was used as a case study. Every section of each chapter in this book is self-contain and can be read it independently. Most of the sections are already published or submitted for publication.

1.3 References

[1] M. Aguilar, J.L. Cortina, Solvent Extraction and Liquid Membranes Fundamentals and Applications in New Materials, 1st ed., Taylor & Francis Group, London, 2008.

[2] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[3] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications, 3rd ed., Elsevier, Amsterdan, 2003.

[4] J.A. Moulijn, A. Stankiewicz, J. Grievink, A. Górak, Process intensification and process systems engineering: A friendly symbiosis, Comput. Chem. Eng. 32 (2008) 3–11.

[5] J.M. Woodley, M. Bisschops, A.J.J. Straathof, M. Ottens, Future directions for in-situ product removal (ISPR), J. Chem. Technol. Biotechnol. 83 (2008) 121–123. doi:10.1002/jctb.1790.

[6] H.-J. Huang, S. Ramaswamy, U.W. Tschirner, B.V. Ramarao, A review of separation technologies in current and future biorefineries, Sep. Purif. Technol. 62 (2008) 1–21.

[7] C.S. López-Garzón, A.J.J. Straathof, Recovery of carboxylic acids produced by fermentation, Biotechnol. Adv. 32 (2014) 873–904. doi:10.1016/j.biotechadv.2014.04.002.

[8] S.T. Yang, C. Lu, Extraction-Fermentation Hybrid (Extractive Fermentation), in: Sep. Purif. Technol. Biorefineries, 1st ed., John Wiley & Sons, 2013: pp. 409–437.

[9] L.M. Vane, Separation technologies for the recovery and dehydration of alcohols from fermentation broths, Biofuels, Bioprod. Biorefining. 2 (2008) 553–588.

[10] P. Yao, Z. Xiao, C. Chen, W. Li, Q. Deng, Cell growth behaviors of Clostridium acetobutylicum in a pervaporation membrane bioreactor for butanol fermentation, Biotechnol. Appl. Biochem. 63 (2016) 101–105. doi:10.1002/bab.1318.

[11] P. Pal, R. Kumar, D. VikramaChakravarthi, S. Chakrabortty, Modeling and simulation of continuous production of L (+) glutamic acid in a membrane-integrated bioreactor, Biochem.

Eng. J. 106 (2016) 68–86. doi:10.1016/j.bej.2015.11.008.

[12] P.A. Sosa, C. Roca, S. Velizarov, Membrane assisted recovery and purification of bio-based succinic acid for improved process sustainability, J. Memb. Sci. 501 (2016) 236–247. doi:10.1016/j.memsci.2015.12.018.

[13] C. Miller, A. Fosmer, B. Rush, T. McMullin, D. Beacom, P. Suominen, Industrial Production of Lactic Acid, in: Ref. Modul. Life Sci., Elsevier, 2017: pp. 179–188. doi:10.1016/B978-0-12-809633-8.09142-1.

[14] J. Vijayakumar, R. Aravindand, T. Viruthagiri, Recent Trends in the Production, Purification and Application of Lactic Acid, Chem. Biochem. Eng. Q. 22 (2008) 245–264. https://hrcak.srce.hr/24811.

[15] A. Komesu, M.R. Wolf Maciel, R. Maciel Filho, Separation and Purification Technologies for Lactic Acid – A Brief Review, BioResources. 12 (2017) 6885–6901. doi:10.15376/biores.12.3.6885-6901.

[16] M. Singhvi, T. Zendo, K. Sonomoto, Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects, Appl. Microbiol. Biotechnol. 102 (2018) 5911–5924. doi:10.1007/s00253-018-9092-4.

[17] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[18] N. Tik, E. Bayraktar, Ül. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, J. Chem. Technol. Biotechnol. 76 (2001) 764–768.

[19] E. V. Yurtov, M.Y. Koroleva, Liquid membranes for extraction, Pet. Chem. 54 (2014) 581–594. doi:10.1134/S0965544114080192.

[20] A. M. Sastre, A.. Kumar, S. J. P., S. R. K., Improved techniques in liquid membrane separations: An overview, Sep. Purif. Methods. 27 (1998) 213–298.

[21] B. Sasikumar, G. Arthanareeswaran, A.F. Ismail, Recent progress in ionic liquid membranes for gas separation, J. Mol. Liq. 266 (2018) 330–341. doi:10.1016/j.molliq.2018.06.081.

[22] C.S. Gholap, S. Panja, P. s. Dhami, J. s. Yadav, S.K. Ghosh, Supported Liquid Membrane transport studies of Pu(IV) using OTDA, a novel diamide, J. Environ. Chem. Eng. 7 (2019) 102784. doi:S2213343718307085.

[23] H. Dou, B. Jiang, M. Xu, J. Zhou, Y. Sun, L. Zhang, Supported ionic liquid membranes with high carrier efficiency via strong hydrogen-bond basicity for the sustainable and effective olefin/paraffin separation, Chem. Eng. Sci. 193 (2019) 27–37. doi:10.1016/j.ces.2018.08.060.

[24] N.M. Kocherginsky, Q. Yang, L. Seelam, Recent advances in supported liquid membrane technology, Sep. Purif. Technol. 53 (2007) 171–177.

[25] R.W. Baker, Membrane Technology and Applications, 2nd ed., John Wiley & Sons, Ltd, Chichester, UK, 2004.

[26] G. Breembroek, G. Witkamp, G. Van Rosmalen, Design and testing of an emulsion liquid membrane pilot plant, Sep. Sci. Technol. 10 (2000) 1539–1571.

[27] P. Cserjési, N. Nemestóthy, K. Bélafi-Bakó, Gas separation properties of supported liquid membranes prepared with unconventional ionic liquids, J. Memb. Sci. 349 (2010) 6–11. doi:10.1016/j.memsci.2009.10.044.

[28] L.J. Lozano, C. Godínez, A. P. de los Ríos, F.J. Hernández-Fernández, S. Sánchez-Segado, F.J. Alguacil, Recent advances in supported ionic liquid membrane technology, J. Memb. Sci. 376 (2011) 1–14. doi:10.1016/j.memsci.2011.03.036.

[29] J. Marták, Š. Schlosser, S. Vlčková, Pertraction of lactic acid through supported liquid membranes containing phosphonium ionic liquid, J. Memb. Sci. 318 (2008) 298–310. doi:10.1016/j.memsci.2008.02.064.

[30] M.F. San Roman, E. Bringas, R. Ibañez, I. Ortiz, Liquid membrane technology: fundamentals and review of its applications, J. Chem. Technol. Biotechnol. 85 (2010) 2–10.

[31] S. Bazhenov, A. Malakhov, D. Bakhtin, V. Khotimskiy, G. Bondarenko, V. Volkov, M. Ramdin, T.J.H. Vlugt, A. Volkov, CO2 stripping from ionic liquid at elevated pressures in gas-liquid membrane contactor, Int. J. Greenh. Gas Control. 71 (2018) 293–302. doi:10.1016/j.ijggc.2018.03.001.

[32] T. Sirman, L. Pyle, A.S. Grandison, Extraction of organic acids using a supported liquid membrane., Biochem. Soc. Trans. 19 (1991) 274S.

[33] B. Yordanov, L. Boyadzhiev, Pertraction of citric acid by means of emulsion liquid membranes, J. Memb. Sci. 238 (2004) 191–197.

[34] D. Cascaval, A. Galaction, C. Oniscu, Selective Pertraction of Carboxylic Acids Obtained by Citric Fermentation, Sep. Sci. Technol. 39 (2005) 1907–1925.

[35] R. Juang, R. Huang, Separation of citric and lactic acids in aqueous solutions by solvent extraction and liquid membrane processes, J. Memb. Sci. 136 (1997) 89–99.

[36] M.A. Malik, M.A. Hashim, F. Nabi, Extraction of Metal Ions by ELM Separation Technology, J. Dispers. Sci. Technol. 33 (2012) 346–356.

[37] F. Leal-Calderon, V. Schmitt, J. Bibette, Emulsion science: basic principles, Second Edi, Springer, 2007.

[38] H.C. Ferraz, L.T. Duarte, M. Di Luccio, T.L.M. Alves, A.C. Habert, C.P. Borges, Recent achievements in facilited transport membrane for separation processes, Brazilian J. Chem. Eng. 24 (2007) 101–118.

[39] J. Fontalvo, A.D. Pérez, Membrana Líquida y proceso para realizarlo, Rad. 15-131023, n.d.

[40] Y. Okubo, T. Maki, N. Aoki, T. Hong Khoo, Y. Ohmukai, K. Mae, Liquid-liquid extraction for efficient synthesis and separation by utilizing micro spaces, Chem. Eng. Sci. 63 (2008) 4070–4077. doi:10.1016/j.ces.2008.05.017.

[41] R. Gupta, S.S.Y. Leung, R. Manica, D.F. Fletcher, B.S. Haynes, Hydrodynamics of liquid–liquid Taylor flow in microchannels, Chem. Eng. Sci. 92 (2013) 180–189.

[42] M.T. Kreutzer, F. Kapteijn, J.A. Moulijn, C.R. Kleijn, J.J. Heiszwolf, Inertial and interfacial effects on pressure drop of Taylor flow in capillaries, AIChE J. 51 (2005) 2428–2440.

[43] B. Zheng, J.D. Tice, R.F. Ismagilov, Formation of droplets of alternating composition in microfluidic channels and applications to indexing of concentrations in droplet-based assays, Anal. Chem. 76 (2004) 4977–4982. doi:10.1021/ac0495743.

[44] M.N. Kashid, I. Gerlach, S. Goetz, J. Franzke, J.F. Acker, F. Platte, D.W. Agar, S. Turek,

Internal circulation within the liquid slugs of a liquid-liquid slug-flow capillary microreactor, Ind. Eng. Chem. Res. 44 (2005) 5003–5010. doi:10.1021/ie0490536.

[45] M. Kashid, O. Detraz, M.S. Moya, I. Yuranov, P. Prechtl, J. Membrez, A. Renken, L. Kiwi-Minsker, Micro-batch reactor for catching intermediates and monitoring kinetics of rapid and exothermic homogeneous reactions, Chem. Eng. J. 214 (2013) 149–156. doi:10.1016/j.cej.2012.10.019.

[46] W. Tanthapanichakoon, N. Aoki, K. Matsuyama, K. Mae, Design of mixing in microfluidic liquid slugs based on a new dimensionless number for precise reaction and mixing operations, Chem. Eng. Sci. 61 (2006) 4220–4232. doi:10.1016/j.ces.2006.01.047.

[47] M. Mendorf, D.W. Agar, Scale-up of Capillary Extraction Equipment, Chemie Ing. Tech. 83 (2011) 1120–1124.

[48] R. Chen, Y.Y. Lee, Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass, Appl. Biochem. Biotechnol. 63–65 (1997) 435–448. doi:10.1007/BF02920444.

[49] S.A. Ahmad, S.R. Lone, Hybrid Process (Pervaporation-Distillation): A Review, Int. J. Sci. Eng. Res. 3 (2012) 1–5.

[50] K. Kraemer, A. Harwardt, R. Bronneberg, W. Marquardt, Separation of butanol from acetone–butanol–ethanol fermentation by a hybrid extraction–distillation process, Comput. Chem. Eng. 35 (2011) 949–963.

[51] P. Pal, J. Sikder, S. Roy, L. Giorno, Process intensification in lactic acid production: A review of membrane based processes, Chem. Eng. Process. Process Intensif. 48 (2009) 1549–1559.

[52] L.M. Vane, A review of pervaporation for product recovery from biomass fermentation processes, J. Chem. Technol. Biotechnol. 80 (2005) 603–629.

[53] K. Hetényi, Á. Németh, B. Sevella, Role of pH-regulation in lactic acid fermentation: Second steps in a process improvement, Chem. Eng. Process. Process Intensif. 50 (2011) 293–299. doi:10.1016/j.cep.2011.01.008.

[54] X. Wang, Y. Wang, X. Zhang, H. Feng, T. Xu, In-situ combination of fermentation and electrodialysis with bipolar membranes for the production of lactic acid: continuous

operation., Bioresour. Technol. 147 (2013) 442–8.

[55] O.A. Prado Rubio, S.B. Jørgensen, G.E. Jonsson, Lactic Acid Recovery in Electro-Enhanced Dialysis: Modelling and Validation, Comput. Aided Chem. Eng. 26 (2009) 773–778. doi:10.1016/S1570-7946(09)70129-4.

[56] H. Honda, Y. Toyama, H. Takahashi, Effective lactic acid production by two-stage extractive fermentation, J. Ferment. Bioeng. 79 (1995) 589–593.

[57] M. Matsumoto, In situ Extractive Fermentation of Lactic Acid by Rhizopus oryzae in an Air-lift Bioreactor, Chem. Biochem. Eng. Q. 32 (2018) 275–280. doi:10.15255/CABEQ.2017.1208.

[58] M. Boonmee, O. Cotano, S. Amnuaypanich, N. Grisadanurak, Improved Lactic Acid Production by In Situ Removal of Lactic Acid During Fermentation and a Proposed Scheme for Its Recovery, Arab. J. Sci. Eng. 41 (2016) 2067–2075. doi:10.1007/s13369-015-1824-5.

[59] Y.K. Hong, W.H. Hong, D.H. Han, Application of reactive extraction to recovery of carboxylic acids, Biotechnol. Bioprocess Eng. 6 (2001) 386–394. doi:10.1007/BF02932319.

[60] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702.

[61] V.S. Kislik, Solvent Extraction: Classical and Novel Approaches, 1st ed., Amsterdam, 2012.

[62] A. Labbaci, G. Kyuchoukov, J. Albet, J. Molinier, Detailed investigation of lactic acid extraction with tributylphosphate dissolved in dodecane, J. Chem. Eng. Data. 55 (2010) 228–233. doi:10.1021/je900315r.

[63] A. Krzyzaniak, B. Schuur, A.B. De Haan, Equilibrium studies on lactic acid extraction with N,N-didodecylpyridin-4-amine (DDAP) extractant, Chem. Eng. Sci. 109 (2014) 236–243.

[64] S.C. Lee, Extraction of succinic acid from simulated media by emulsion liquid membranes, J. Memb. Sci. 381 (2011) 237–243. doi:10.1016/j.memsci.2011.07.039.

[65] Q.-Z. Li, X.-L. Jiang, X.-J. Feng, J.-M. Wang, C. Sun, H.-B. Zhang, M. Xian, H.-Z. Liu, Recovery Processes of Organic Acids from Fermentation Broths in the Biomass-Based

Industry, J. Microbiol. Biotechnol. 26 (2016) 1–8. doi:10.4014/jmb.1505.05049.

[66] N. Jusoh, N. Othman, N.A. Nasruddin, Emulsion liquid membrane technology in organic acid purification, Malaysian J. Anal. Sci. 20 (2016) 436–443. doi:10.17576/mjas-2016-2002-28.

[67] N. Harruddin, S.M. Saufi, C.K.M. Faizal, A.W. Mohammad, Removal of acetic acid from aqueous solution by polyethersulfone supported liquid membrane, Chem. Eng. Trans. 56 (2017) 847–852. doi:10.3303/CET1756142.

[68] F. Garavand, S.H. Razavi, I. Cacciotti, Synchronized extraction and purification of L-lactic acid from fermentation broth by emulsion liquid membrane technique, J. Dispers. Sci. Technol. 39 (2018) 1291–1299. doi:10.1080/01932691.2017.1396225.

[69] S. Joachim, P. Wasserscheid, Production of lactic acid by way of fermentation and extraction of amines, US 2010/0273224 A1, 2010. http://www.google.st/patents/US20100273224 (accessed March 23, 2014).

[70] C. Schöller, J. Chaudhuri, D. Phyle, Emulsion liquid membrane extraction of organic acids—I. A theoretical model for lactic acid extraction with emulsion swelling, Chem. Eng. Sci. 47 (1992) 41–48. http://www.sciencedirect.com/science/article/pii/000925099280198L (accessed April 24, 2014).

[71] R.-S. Juang, S.-H. Lee, R.-C. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239. doi:10.1016/S0376-7388(97)00206-8.

[72] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[73] A. Demirci, A.L. Pometto, K.R. Harkins, Rapid screening of solvents and carrier compounds for lactic acid recovery by emulsion liquid extraction and toxicity on Lactobacillus casei (ATCC 11443), Bioseparation. 7 (1999) 297–308.

[74] B.S. Chanukya, M. Kumar, N.K. Rastogi, Optimization of lactic acid pertraction using liquid emulsion membranes by response surface methodology, Sep. Purif. Technol. 111 (2013) 1–8.

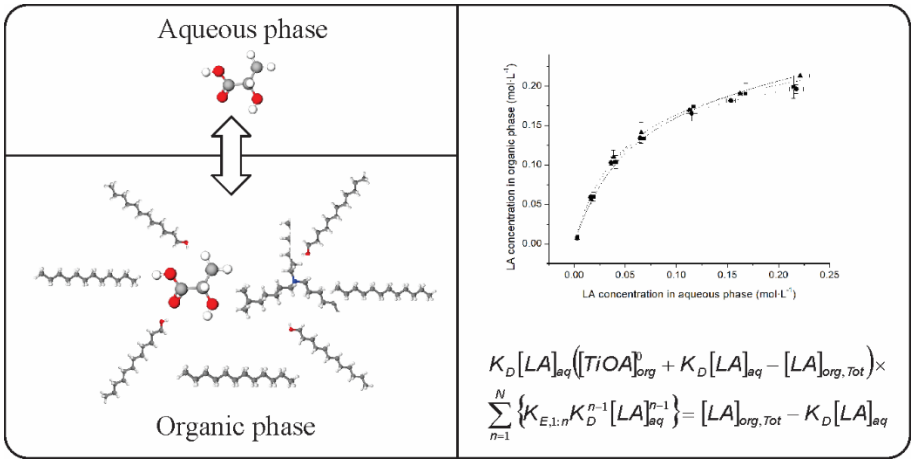
[75] N. Rastogi, B.S. Chanukya, Supported Liquid Membrane Composed of Tertiary or/and

Quaternary Amine for the Extraction of Lactic Acid, Int. J. Membr. Sci. Technol. 2 (2015) 19–28. doi:10.15379/2410-1869.2015.02.02.03.

[76] A. Kumar, A. Thakur, P.S. Panesar, Statistical optimization of lactic acid extraction using Green Emulsion Ionic Liquid Membrane (GEILM), J. Environ. Chem. Eng. 6 (2018) 1855–1864. doi:10.1016/j.jece.2018.01.037.

[77] A. Kumar, A. Thakur, P.S. Panesar, Lactic acid extraction using environmentally benign Green emulsion ionic liquid membrane, J. Clean. Prod. 181 (2018) 574–583. doi:10.1016/j.jclepro.2018.01.263.

2. Chapter 2: Liquid-liquid equilibria of potential liquid membranes for lactic acid removal



2.1 Liquid-liquid equilibria for trioctylamine/1-dodecanol/lactic acid/water system at 306.1, 310.1 and 316.1 k: experimental data and prediction[1]

Abstract

Liquid-liquid equilibria of aqueous solutions of lactic acid with trioctylamine diluted in 1-dodecanol was measured experimentally at three temperatures (306.1, 310.1 and 316.1 ±0.1 K). During the transfer process, lactic acid reacts with trioctylamine to produce an amine-lactate complex. Two models were proposed to predict the liquid-liquid equilibria. The first model considered the ratio of chemical equilibrium concentration and the distribution coefficient. Those parameters have been determined by fitting the experimental data. It was found that as temperature increases, the distribution coefficient increases and equilibrium constant decreases. The second proposed model involved the Non-Random two liquid activity model. Energies of binary interaction and equilibrium constant were fitted to experimental data. The equilibrium constant and partition coefficients show the same trends that the first model, however the first model shows a better prediction as compared to the second model of the liquid-liquid equilibrium data. These two models are especially suitable at low lactic acid concentrations in the aqueous phase where the experimental standard deviation is low.

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2.1.1 Introduction

Lactic acid (LA) is an important product due to its applications in food (as food additive and preservative), and in chemical, cosmetic and pharmaceutical industries. Nowadays interest in LA production and recovery, for instance from fermentation broths, is growing due to the potential production of biodegradable and biocompatible polymers, mainly polylactic acid [1–3].

On the other hand, reactive equilibria systems have attracted significant attention, specifically in extraction of carboxylic acids. LA recovery has been studied using different technologies including liquid extraction [1,4–6] and liquid membranes [7–9]. In these separation process, tertiary amines as trialkylamine (TAA), trioctylamine (TOA), alamine 336 have been often used for removal of LA and other organic acids [1,4,17,5,10–16]. Alamine has been used as extractant with different solvents as 1-dodecanol, 1-decanol, 1-hexanol, phenylethanol and cyclohexanol to remove pyruvic acid [11,15]. TAA in a mix of 1-octanol/n-heptane was studied to extract lactic, malic and citric acids [5]. TOA has been tested in mixtures of decanol, dodecane, 1-octanol for removal of LA from aqueous solutions [1,5,13,18].

Several liquid-liquid equilibria (LLE) models for systems containing carboxylic acids and tertiary amines have been proposed. A method based on chemical modeling that involved a thermodynamic extraction constant [5], mass balances and a dissociation constant was suggested to predict LLE of lactic, malic and citric acids in amine solutions [5]. In other studies, mass action law of equilibria, apparent equilibrium constant and the amine concentration were used to predict LLE of carboxylic acids such as lactic, acetic, propionic and butyric [6,10,12]. A similar study, that included mass action law, the distribution coefficient and Henderson-Hasselbalch equation were used to model the LLE for pyruvic acid with TOA in 1-octanol system [15]. SERLAS model based on solvation energy relationship was proposed to predict the LLE for mixtures of water, pyruvic acid, alcohol and alamine [11]. Juang & Huang [13] proposed a model based on distribution ratios and equilibrium constants, and the LA dissociation constant in water to predict equilibria data of reactive extraction of LA with an amine extractant. Probably, the most detailed LLE model involved a method of Gibbs energy minimization using a flash algorithm, developed by Großmann, combined with the modified Pitzer and Debye-Hückel equations of ionic species [14,16,17]. In this study, an infrared spectroscopy technique was used to obtain information about the stoichiometry of the complex formation.

In this work, the experimental values of the reactive liquid-liquid equilibria of TOA/1-Dodecanol/Water/Lactic acid system are presented at 306.1, 310.1 and 316.1 K. In this system there

is a chemical reaction between lactic acid and TOA to produce an amine-lactate complex. This complex promotes the separation and provides a higher separation degree than using only the organic solvent. Two models were proposed to predict the reactive liquid-liquid equilibria. The first model considers the distribution coefficient, the equilibrium constant and the material balances. The prediction of this model gives an appropriate description of the liquid-liquid equilibria experimental data. The second model involves an activity coefficient model and it results in a more complex to fit set of equations, due to the amount of parameters to estimate and the nonlinearity of the model.

2.1.2 Experimental section

Materials

Trioctylamine and 1-dodecanol for synthesis were supplied by Merck Millipore. Both reagents are colorless and practically water insoluble (0.0001 and 0.004 $g \cdot L^{-1}$ at 298.15 K respectively). L(+)-lactic acid were supplied by Panreac Química S.A.U. (assay 88.0-92.0%). The purity of lactic acid was assessed by titration with NaOH of Carlo Herba (assay ≥ 97.0%) using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). Aqueous solutions of lactic acid (150 $g \cdot L^{-1}$) were heated at 363.1 K under total reflux for 12 hours for dimmers hydrolysis [1,3]. Water HPLC grade was used for all aqueous solutions.

Experimental Procedure and Analysis

Experimental LLE was carried out at three temperatures (306.1, 310.1 and 316.1 K) with 16 samples for each temperature. The two proposed models in this paper contain 2 and 15 parameters, respectively. Thus, 16 experimental points were used to fit the corresponding parameters in these models. All liquid−liquid equilibrium experiments were carried out in 1.5 ml vials. At the beginning of each experiment, every vial contained 0.4 ml of organic phase (0.8 $mol \cdot L^{-1}$ of TOA in 1-dodecanol) and 0.4 ml of aqueous phase (with LA concentrations between 10 and 150 $g \cdot L^{-1}$). The LA concentrations in the aqueous phase were selected based on the final concentration of LA in fermentation broths [2,19–21]. The TOA concentration in the organic phase was selected based on the studies of Juang [8] for LA removal with a supported liquid membrane using TOA. And 1-dodecanol was selected due to its lower water solubility than that of alcohols and alkanes of smaller carbon chain. The experimental protocol was performed at constant temperature and consisted of three steps: agitation, decantation and sampling. Firstly, each vial was shaken during 72 hours at 180 rpm in a shaking water bath (Boekel Scientific) with a reciprocating shaking (±0.1 °C). Then, the samples were decanted (stabilization step) for 72 hours in a GC oven (ChromPack with ±0.1 °C).

Afterwards, the LA residual concentration in the aqueous phase was measured by HPLC (Elite LaChrom), using ORH-801 column (Transgenomic®) and RI detector at 308 K. As a mobile phase, 0.01 N H_2SO_4 solution was used at 0.8 $ml \cdot min^{-1}$ flow rate. By using the mass balances the corresponding total LA concentrations in the organic phase were calculated. The volume of the organic and aqueous phases were assumed constant that according to our calculations introduce a maximal error of ±0.9%. However, Sabalová *et al.* [22] have calculated a maximal error of ±3% for butyric acid using several solvents with TOA.

Distribution coefficient was measured at the highest LA concentration in the aqueous phase at the three temperatures following the aforementioned procedure. For the organic phase 1-dodecanol was used. The equilibria concentration of LA in the aqueous phase was measured by HPLC and in the organic phase by titration using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand).

2.1.3 Theoretical section

The liquid-liquid equilibria system consists of TOA and 1-dodecanol in the organic phase, and lactic acid in the aqueous phase. The mass transfer of organic compounds toward the aqueous phase was neglected due to the low solubility of TOA and 1-dodecanol in water (Merck material safety data sheets - MSDS). It was observed that for alcohols increasing the carbon chain length, water solubility decreases being 1-dodecanol one of the alcohols with a high hydrophobicity [11]. The water solubility, with and without LA in 1-dodecanol and TOA/1-dodecanol, were measured in this paper using Karl-Fischer titration to obtain values of 3.96% ±0.05, 3.03% ±0.05, 4.98% ±0.22 and 1.75% ±0.20 w/w, respectively at 310.5 K. Consequently, in this work the water mass transfer between the phases is not taken into account in the reported data and also LA is considered as the only compound that is transferred between the liquid phases.

The LA in the organic phase comes from two contributions: free LA that is soluble in the organic phase and LA that reacts with TOA at the interface and in the bulk of the organic phase to produce an amine-lactate complex according to equation 1. Most of the lactic acid in the organic phase is mainly due to chemical reaction because the solubility of LA in 1-dodecanol is low. Nevertheless, both contributions are included in the models that are presented below.

$$\sigma TOA + \phi LA \Leftrightarrow (LA)_{\phi}(TOA)_{\sigma} \quad (1)$$

Two liquid-liquid equilibria models were proposed. The first model uses the material balances, the distribution coefficient and the reaction equilibrium constant. The reaction equilibrium constant is

molar concentration based, and generally it is called concentration chemical equilibrium ratio [23] or apparent equilibrium constant [6,10,24]. The second model takes into account the activities of the species involved in the system and the chemical equilibrium constant based on activity coefficients.

Model based on equilibrium constant and distribution coefficient

This liquid-liquid equilibria model involves equation 1 and a value of the reaction stoichiometric ratio. Some researchers have found stoichiometric ratios (TOA:LA) of (1:1), (1:2), and (2:3) [5,9] or (1:1), (1:2) and (1:3) [24,25]. Experimentally this ratio was calculated in this paper with the free LA, LA-TOA complex and total TOA concentrations at equilibrium in the organic phase using the experimental data where the total LA concentration in the organic phase at each temperature is maximum. A simple equation of the liquid-liquid equilibria for this system can be developed from the equilibrium constant (eq. 2) with a stoichiometric ratio of (1:1), a distribution coefficient (eq. 3), the LA mass balance in the organic phase (eq. 4) taking into account the TOA mass balance (eq. 5), however, the followed model can be derived to several stoichiometric ratios (1:ϕ). The stoichiometric ratio was estimated from the experimental data as it is shown below.

$$K_E = \frac{[LA-TOA]_{org}}{[LA]_{org}[TOA]_{org}} \tag{2}$$

$$K_D = \frac{[LA]_{org}}{[LA]_{aq}} \tag{3}$$

$$[LA]_{org,Tot} = [LA]_{org} + [LA-TOA]_{org} \tag{4}$$

$$[TOA]_{org} = C^0_{TOA} - [LA-TOA]_{org} \tag{5}$$

Combining equations 2-5, the following expression can be obtained:

$$[LA]_{org.Tot} = \frac{K_D^2 K_E [LA]_{aq} + K_D K_E C^0_{TOA} + K_D}{K_D K_E + 1/[LA]_{aq}} \tag{6}$$

K_E and K_D were calculated at each temperature by minimizing the sum of squares of the deviations between the experimental and predicted LA concentrations in the organic phase (eq. 7). It was used *fmincon* and *globalsearch* of Matlab® with the interior point algorithm and a routine that find the optimum value of the error with a global minimum.

$$\delta_{\min} = \sum \left([LA]^{pred}_{org} - [LA]^{\exp}_{org}\right)^2 \tag{7}$$

If the enthalpy and entropy of the complexation reaction are assumed to be constant over the short temperature range [24,25] as occurs in this work, the Van't Hoff's equation can be used as follows, where the enthalpy and entropy change of reaction can be obtained:

$$\ln\left(K_E\right) = -\frac{\Delta H_{rxn}}{RT} + \frac{\Delta S}{R} \tag{8}$$

Model based on NRTL with chemical reaction

This model was developed taking into account the material balance, phase equilibria and the equilibrium equations for both liquid phases, under the condition that the LA is the only substance that is transferred from the aqueous phase to the organic phase.

The molar balance includes the chemical reaction (for TOA and LA in the organic phase) and the LA transferred from the aqueous phase to the organic phase (eq. 9-12).

$$L_1 \cdot x_{LA}^{I} + L_2 \cdot x_{LA}^{II} + L_2 \cdot x_{LA-TOA}^{II} = F \cdot z_{LA} \tag{9}$$

$$L_1 \cdot x_{H_2O}^{I} = F \cdot z_{H_2O} \tag{10}$$

$$L_2 \cdot x_{1DC}^{II} = F \cdot z_{1DC} \tag{11}$$

$$L_2 \cdot x_{TOA}^{II} + L_2 \cdot x_{LA-TOA}^{II} = F \cdot z_{TOA} \tag{12}$$

Phase equilibrium is given by equation 13:

$$\gamma_{LA}^{I} \cdot x_{LA}^{I} = \gamma_{LA}^{II} \cdot x_{LA}^{II} \tag{13}$$

And the equilibrium equation of the reaction as a function of activities is given by:

$$K_{eq} = \left(\frac{x_{LA-TOA}^{II} \gamma_{LA-TOA}^{II}}{\left(x_{TOA}^{II} \gamma_{TOA}^{II}\right)^{\sigma} \left(x_{LA}^{II} \gamma_{LA}^{II}\right)^{\phi}} \right) \tag{14}$$

The proposed model, using NRTL activity coefficient model (eq. 15), requires fifteen binary interaction energies that are unknowns (eq. 16 - 17).

$$\ln(\gamma_i) = \left[\frac{\sum_{j=1}^{N} \tau_{ji} G_{ji} x_j}{\sum_{k=1}^{N} G_{ki} x_k} + \sum_{j=1}^{N} \frac{x_j G_{ij}}{\sum_{k=1}^{N} G_{kj} x_k} \left(\tau_{ij} - \frac{\sum_{l=1}^{N} x_l \tau_{lj} G_{lj}}{\sum_{k=1}^{N} G_{kj} x_k} \right) \right] \quad (15)$$

$$\tau_{ji} = \left(g_{ij} - g_{ii}\right) / RT \quad (16)$$

$$G_{ji} = \rho_{ji} \exp\left(-\alpha_{ji} \tau_{ji}\right) \quad (17)$$

These energy parameters were fitted using the experimental data. Values of the reaction equilibrium constant at each temperature from the first model were used as initial values in the optimization process. The α_{ij} parameters values were selected in agreement with the categories described by Prausnitz [26] and are presented in Table 1.

Table 1. Nonrandomness constant for binary *ij* interactions for the TOA/1-Dodecanol/LA/water system[26].

α_{ij}	**LA**	**TOA**	**1DC**	**LA-TOA**	**H_2O**
LA	1	0.3	0.4	0.3	0.4
TOA	0.3	1	0.32	0.23	0.37
1DC	0.4	0.32	1	0.39	0.42
LA-TOA	0.3	0.23	0.39	1	0.4
H_2O	0.4	0.37	0.42	0.4	1

The g_{ij} parameters were fitted at each temperature using a minimization of the following error square sum, based on LA partition coefficient[14].

$$EQS = \sum_{m}^{N} \sum_{n}^{Q} \left(\frac{\mathrm{K}_m^{\exp} - \mathrm{K}_m^{cal}}{\mathrm{K}_m^{\exp}} \right)_n^2 \quad (18)$$

$$\mathrm{K}_m = x_m^{org} / x_m^{ac} \quad (19)$$

To estimate the g_{ij} parameters and K_{eq}, the sum square error in the equation 18 was used as minimization function using *fminsearch* of Matlab®.

2.1.4 Results and discussion

Experimental LLE Data

The equilibrium experimental data at each temperature are shown in the tables 2-4. In the organic phase, LA molar fraction corresponds to the total concentration of LA (free and complex form). The molar fraction deviation was calculated as error propagation [27] taking into account LA initial concentration deviation (by HPLC) and volumetric measurement error with an automatic pipet.

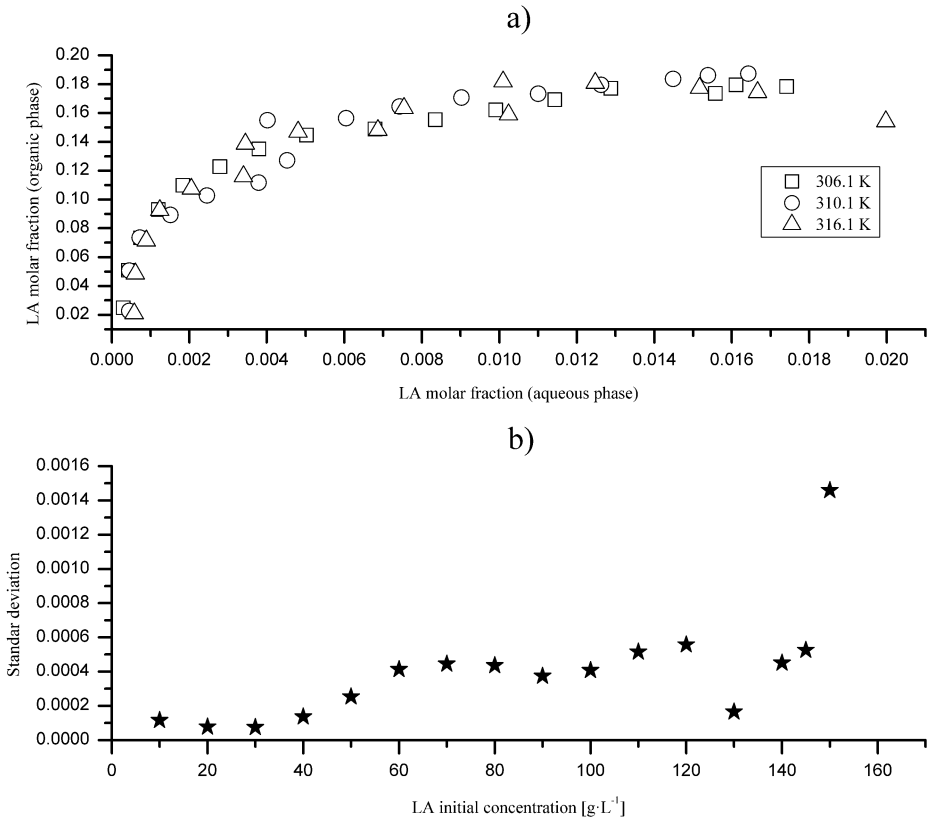


Figure 1. a) Experimental liquid-liquid equilibria for the TOA/1-Dodecanol/LA/water system at three different temperatures and b) experimental standard deviation as function of the LA concentration in the aqueous phase.

Figure 1a shows the liquid-liquid equilibria experimental molar fractions of lactic acid at three temperatures and Figure 1b displays the standard deviation ($s^2 = 1/n*\Sigma(x_i-\underline{x})$, where n is the data number, x_i are the experimental data and $\underline{x}$ is the mean of the experimental data) for the three temperatures, with a maximum deviation of 0.00146. The standard deviations were calculated by using the experimental data at the three temperatures for each LA concentration in the aqueous phase

in order to compare the experimental results among temperatures as it is shown in Figure 1b. The largest standard deviation corresponds to the higher LA concentration. The experimental data at 306.1 and 310.1 K have the same trend, except one point at 310.1 K with a LA molar fraction of 0.1550 in the organic phase and the cause of the deviation in this point is unknown for us. The experimental data at 316.1 K have a clear trend at low LA concentrations, but the data scatter increases at higher concentrations and its trend is less clear (specially the point at the highest LA concentration). This may be due to water evaporation that increases as the temperature rises and the high LA concentrations. The vial is a closed container however vapor losses can happen because the containers were closed manually.

Table 2. Measured liquid-liquid equilibria molar fractions for the TOA/1-Dodecanol/LA/water system at 306.1 K.

Aqueous phase	**Organic phase**		
x_{LA}	x_{LA}	x_{1DC}	x_{TOA}
Equilibrium data at 306.1 K			
0.0003	0.0249	0.7643	0.2107
0.0004	0.0509	0.7440	0.2051
0.0007	0.0733	0.7264	0.2003
0.0012	0.0930	0.7110	0.1960
0.0018	0.1099	0.6978	0.1924
0.0028	0.1229	0.6876	0.1896
0.0038	0.1350	0.6780	0.1869
0.0050	0.1447	0.6704	0.1848
0.0068	0.1489	0.6671	0.1839
0.0084	0.1555	0.6620	0.1825
0.0099	0.1621	0.6568	0.1811
0.0114	0.1692	0.6512	0.1795
0.0129	0.1772	0.6450	0.1778
0.0156	0.1736	0.6478	0.1786
0.0161	0.1795	0.6432	0.1773
0.0174	0.1784	0.6441	0.1776

[a]Standard uncertainties $u(x)$=0.00146, $u(T)$=0.1K.

High LA concentrations in the organic phase, corresponding to LA molar fractions higher than 0.15 at each temperature, were used to calculate the equilibrium constant. In these experimental results, the molar ratio TOA and total LA in the organic phase (at the maximum value) corresponds to 1.02, 1.06, and 1.23 at 306.1 K, 310.1 K and 316.1 K respectively. These results indicate that for the low

LA concentrations used in our experiments the stoichiometry ratio (TOA:LA) is 1:1. Perhaps, for higher LA concentrations the TOA:LA ratio could be different.

Table 3. Measured liquid-liquid equilibria molar fractions for the TOA/1-Dodecanol/LA/water system at 310.1 K.

Aqueous phase	**Organic phase**		
x_{LA}	x_{LA}	x_{1DC}	x_{TOA}
Equilibrium data at 310.1 K			
0.0005	0.0227	0.7661	0.2112
0.0005	0.0506	0.7442	0.2052
0.0007	0.0736	0.7262	0.2002
0.0015	0.0893	0.7139	0.1968
0.0025	0.1028	0.7033	0.1939
0.0038	0.1117	0.6963	0.1920
0.0045	0.1272	0.6842	0.1886
0.0040	0.1550	0.6624	0.1826
0.0060	0.1565	0.6612	0.1823
0.0074	0.1646	0.6549	0.1805
0.0090	0.1707	0.6501	0.1792
0.0110	0.1733	0.6480	0.1787
0.0126	0.1795	0.6432	0.1773
0.0145	0.1837	0.6399	0.1764
0.0154	0.1861	0.6380	0.1759
0.0164	0.1873	0.6370	0.1756

[a]Standard uncertainties $u(x)$= 0.00146, $u(T)$=0.1K.

Also a small amount of free LA was calculated that corresponds to LA that is soluble in the organic phase. Several studies have shown that LA solubility in 1-dodecanol is low [11], therefore this amount of free LA must be low. The maximum calculated free lactic acid concentrations in the organic phase were calculated using equation 3 and the total amount of LA in the organic phase to obtain 0.069, 0.155 y 0.260 mol·L^{-1} at 306.1 K, 310.1 K and 316.1 K, respectively. The calculated values of K_D are shown below (Table 5).

Table 4. Measured liquid-liquid equilibria molar fractions for the TOA/1-Dodecanol/LA/water system at 316.1 K.

Aqueous phase	Organic phase		
x_{LA}	x_{LA}	x_{1DC}	x_{TOA}
Equilibrium data at 316.1 K			
0.0006	0.0209	0.7675	0.2116
0.0006	0.0485	0.7458	0.2056
0.0009	0.0715	0.7279	0.2007
0.0012	0.0926	0.7113	0.1961
0.0021	0.1074	0.6997	0.1929
0.0034	0.1161	0.6929	0.1910
0.0034	0.1387	0.6752	0.1861
0.0048	0.1469	0.6687	0.1844
0.0069	0.1482	0.6678	0.1841
0.0075	0.1635	0.6557	0.1808
0.0102	0.1589	0.6593	0.1818
0.0101	0.1817	0.6414	0.1768
0.0125	0.1809	0.6421	0.1770
0.0152	0.1774	0.6448	0.1778
0.0167	0.1744	0.6472	0.1784
0.0200	0.1542	0.6630	0.1828

[a]Standard uncertainties $u(x)$= 0.00146, $u(T)$=0.1K.

Table 5. Distribution coefficients, equilibrium constant and fitting deviation (eq 18) for the TOA/1-Dodecanol/LA/water system using the model based on the equilibrium constant and distribution coefficient.

Temperature (K)	K_D	K_E	Sum of square error	K_D (exp)[b]
306.1	0.0809	152.8	0.0066	0.0816 ± 0.0003
310.1	0.1920	49.2	0.0407	0.0916 ± 0.0007
316.1	0.2627	34.5	0.0272	0.1060 ± 0.0007

[a]Standard uncertainties $u(T)$=0.1K. [b]Experimental values in 1-dodecanol at 150 g·L^{-1} of LA in the aqueous phase.

Data fit model based on the equilibrium constant and distribution coefficient

Figure 2, shows the obtained data fit at the three temperatures. At 316.15 K the values with a high scatter were not taken into account (0.1589, 0.1809, 0.1774, 0.1744 and 0.1542 LA molar fraction in the organic phase). Also, Figure 3 shows the fitted values of distribution coefficients and the

equilibrium constant as function of temperature. These values are also included in Table 5 where the corresponding fitting deviations are shown.

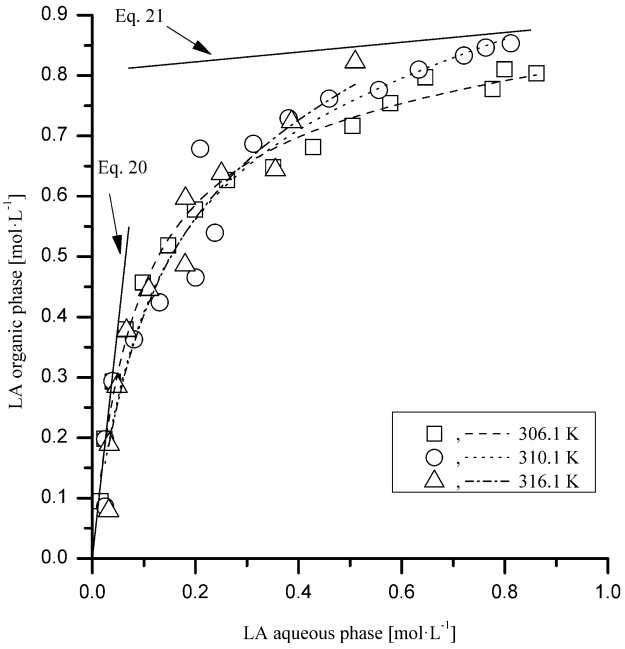


Figure 2. Liquid-liquid equilibria and model fit for the TOA/1-Dodecanol/LA/water system using the model based on equilibrium constant and distribution coefficient. Symbols represent experimental data and discontinuous lines the fitted model.

The distribution coefficient increases as temperature rises (Table 5, Figure 3a), due to higher solubility of LA as expected. The higher the temperature, the higher the fitting deviation will be. However, the error fit at 316.1 K is below than 310.1 K, due to 316.1K the scatter values were not considered for fitting. Experimental K_D values (Table 5) linearly increase with increasing. A good agreement between the fitted and the measured values of K_D was found at 306.1 K. However, experimental K_D values have a lower dependence of temperature than the fitted values, perhaps because in the last case there is not TOA in the organic phase. If TOA is present in the organic phase, a solvation of TOA-LA complex on free LA can improve LA solubility. There is not good agreement between experimental and predicted ELL data if the experimental values of K_D are used in eq 6 (not shown). In this case, the predicted correlations of LA in the organic phase become less sensitive to temperature than measured values shown in Figure 1a.

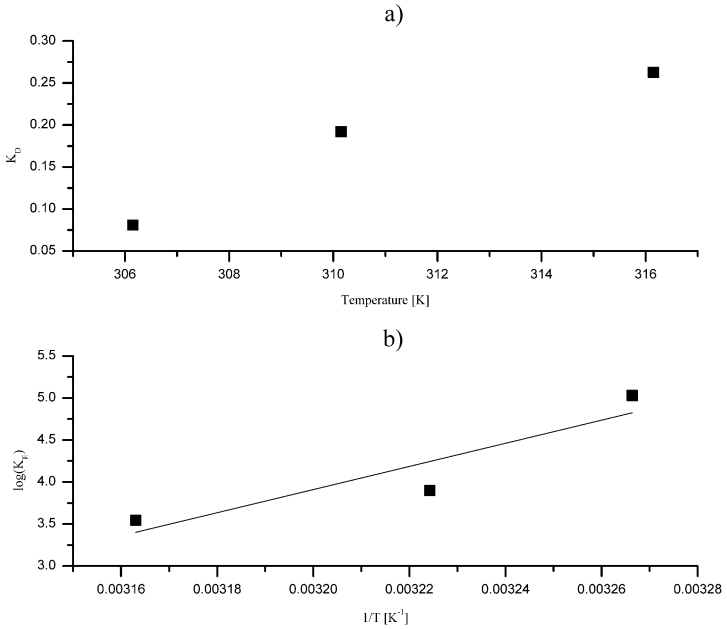


Figure 3. a) Temperature effect on the distribution coefficient. b) Van't Hoff's fit to determine the values of enthalpy change of reaction and entropy (TOA/1-Dodecanol/LA /water system). Data calculated from the model based on equilibrium constant and distribution coefficient.

The values of heat of reaction and entropy change of reaction were estimated from the slope and intercept of the straight line in the Figure 3b. The equilibrium constant decreases as temperature rises, being an exothermic reaction (ΔHrxn = -27.36 kcal·mol^{-1}). The nature of this reaction is due to the complexation reaction in the organic phase that involves a proton-transfer or hydrogen bond [25]. The entropy change was estimated as -0.0798 kcal·(mol·K)$^{-1}$.

In agreement with equation 6 and the results of Figure 2, two limits cases can be analyzed. For low and high LA concentration in the aqueous phase equations 20-21 can be obtained from equation 6, respectively.

$$[LA]_{org,Tot} = \left(K_E C_{TOA}^0 + 1\right) K_D [LA]_{aq} \tag{20}$$

$$[LA]_{org,Tot} = K_D [LA]_{aq} + \left(C_{TOA}^0 + 1/K_E\right) \tag{21}$$

At low LA concentrations, independently of the temperature, all LA molecules react with TOA molecules to produce an amine-lactate complex, due to an excess of TOA. These values of LA

concentrations in the organic phase are described by equation 20 as it is shown in Figure 2. The effect of temperature on the LA concentration in the organic phase at low LA concentrations is not significant, and it can be observed in Figure 2 with the predicted data (solid discontinuous lines). Based on equation 20, the LA concentration ratio between the organic and the aqueous phases is directly proportional to K_D and K_E. Although, K_D increases and K_E decreases as the temperature rises the effect of temperature on K_E is more significant and thus the LA concentration in the organic phase slightly decreases as the temperature rises at low LA concentrations in the aqueous phase.

At high LA concentrations, LA is in excess for a stoichiometric ratio (TOA:LA) of 1:1 and most of the LA molecules react with TOA. The remaining LA is solubilized as free acid in the organic phase as it is described by equation 21 (Figure 2). Equation 21 shows that the LA concentration in the organic phase increases as the LA concentration in the aqueous phase rises for high LA concentrations in this phase. If, in equation 21, K_D is zero (no solubility of LA in the organic phase) the total LA concentration in the organic phase would be independent of the LA concentration in the aqueous phase. On the other hand, if K_E is zero (without chemical reaction) the total LA concentration in the organic phase will be proportional to the lactic acid concentration in the aqueous phase (from eq. 6).

Model based on NRTL with chemical reaction

The calculated LA concentrations in the organic phase using this model are presented in Figure 4 where the fitted K_{eq} values are 25.9049, 19.7178 and 11.9151 at 306.1 K, 310.1 K and 316.1 K respectively. The calculated equilibrium constants decrease as temperature increases. This trend is in accordance with results of the first model, however the values differ. The prediction of the model at low aqueous LA concentration is better than at high LA concentrations because, as it was shown above, the experimental standard deviation of the LA concentration in the organic phase increases as the LA concentration rises in the aqueous phase. The binary interaction parameters (g_{ij} in the eq. 16) are summarized in Table 6.

Table 6. Energies of interaction between an *i-j* pair molecules, obtained from data fit for the TOA/1-Dodecanol/LA/water system using the model based on NRTL with chemical reaction.

g_{ij}	LA	TOA	1DC	LA-TOA	H_2O
LA	1768.12	286.798	85.8743	880	774.239
TOA	286.798	631.92	2568	565.924	657.285
1DC	85.8743	2568	1320.41	538.445	731
LA-TOA	880	565.924	538.445	1686.7	857.265
H_2O	774.239	657.285	731	857.265	1064

The g_{ij} parameters and K_{eq} were used to calculate the partition coefficient as function of activity as it is shown in equation 22.

$$\frac{x_{LA}^{II}}{x_{LA}^{I}} = K = \frac{\gamma_{LA}^{I}}{\gamma_{LA}^{II}} \tag{22}$$

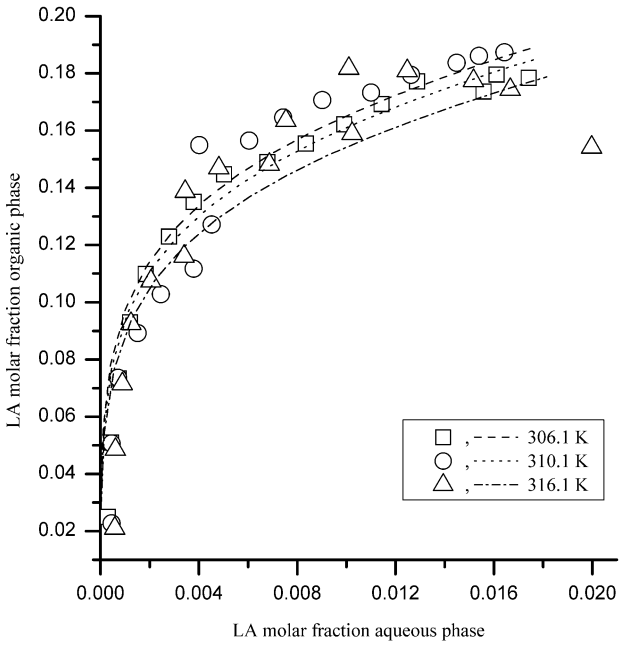


Figure 4. Liquid-liquid equilibria and model fit for the TOA/1-Dodecanol/LA/water system using a model based on NRTL. Symbols represent experimental data and discontinuous lines the fitted model.

The partition coefficient was calculated at each concentration of equilibrium and at three temperatures. These results (Figure 5) show that the coefficient distribution is approximately constant at each temperature. However, at low LA concentrations the *K* values are higher than at high LA concentrations. In order to compare these results, the ratio between K_D and *K* can be estimated using equation 23.

$$K = K_D \frac{C_{Tot}^{aq}}{C_{Tot}^{org}} \tag{23}$$

A single K_D value was calculated at each temperature from the first model. Also K_D values, calculated with equation 23, were calculated for the second model (NRTL model) as function of LA concentration at each temperature. The calculated K_D values in the second model were practically constant, similar to the K values shown in the Figure 5. To compare K_D values between the two models, the mean K_D values were used from the second model. According to the values of K_D in the first model, the following K values were obtained: 0.9846, 0.9813 and 0.9858 at 306.1, 310.1 and 316.1 K, respectively. In the first model the partition coefficients are approximately one order of magnitude higher than those in the second model. Nevertheless, the data fit from the first model was in a better agreement with experimental data than the second proposed model. These two models are especially suitable at low lactic acid concentrations in the aqueous phase where the experimental standard deviation is low.

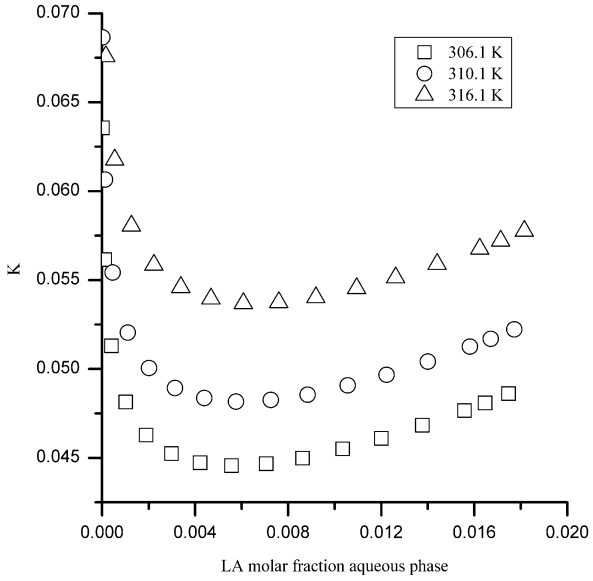


Figure 5. Partition coefficients as function of LA concentration in the aqueous phase and temperature calculated by the NRTL model.

2.1.5 Conclusions

Liquid-liquid equilibria for the trioctylamine/1-dodecanol/lactic acid/water system at 306.1, 310.1 and 316.1 K was experimentally measured and two models are proposed to predict the equilibria data. The liquid-liquid equilibria model based on a reaction equilibrium constant and a distribution coefficient is simple to develop and the fit is in agreement with the experimental data. The Non-

Random Two Liquid thermodynamic model with chemical reaction is in agreement with experimental data at LA molar fraction lower than 0.15 with a sum of least squares smaller than 2.6×10^{-3} in the range of temperatures and concentrations evaluated. However, the model based on equilibrium constant and distribution coefficient shows the best prediction trend curve in the whole range of lactic acid concentrations. Both models are suitable for predicting the lactic acid concentration in the organic phase especially at low lactic acid concentrations in the aqueous phase. The experimental standard deviation of the taken data increases as the lactic acid concentration in the aqueous phase rises.

Experimental value of K_D at 306.1 K is in agreement with K_D obtained by fitting from the model based on an equilibrium constant of reaction and a distribution coefficient. For both models, as temperature increases the distribution coefficient and partition coefficient rise. It was found that the reaction to produce the amine-lactate complex is exothermic (-27.36 kcal·mol^{-1}) and the equilibrium constant decreases as the temperature rises. Additionally, it was found a enthalpy change of reaction of -0.0798 kcal·(mol·K)$^{-1}$.

NOTATION

C	Molar concentration [mol·L^{-1}]
C^0_{TOA}	TOA initial concentration
F	Total initial mol
g_{ij}	Energies of interaction between an *ij* pair of molecules
G_{ij}	Coefficient as defined in equation 17
K	Partition coefficient (eq. 19)
K_D	Distribution coefficient (eq. 3)
K_E	Concentration chemical equilibrium ratio [L·mol^{-1}] (eq. 2)
K_{eq}	Chemical equilibrium constant
L	Total mol in equilibria
R	Gas constant [Kcal·(K·mol)$^{-1}$]
T	Temperature [K]
x	Molar fraction in equilibria
z	Initial molar fraction (global)

Greek letters

α_{ij}	Nonrandomness constant for binary *ij* interactions
δ_{min}	Sum of squares deviations
ΔH_{rxn}	Enthalpy change on reaction [Kcal·mol]

ΔS	Entropy change on reaction [Kcal·(K·mol)$^{-1}$]
γ	Activity coefficient
ϕ	Stoichiometric coefficient to LA
ρ_{ij}	coefficient = 1 for NRTL
τ_{ij}	Coefficient defined in equation 16
σ	Stoichiometric coefficient to TOA

Subscripts and superscripts

I	Phase I (aqueous)
II	Phase II (organic)
1DC	1-dodecanol
aq	Aqueous phase
exp	Experimental
H_2O	Water
i	Component *i*
LA	Lactic acid
LA-TOA	Complex amine-lactate
m	Number of substances
n	Data number
org	Organic phase
pred	Predicted
TOA	Trioctylamine
Tot	Total

2.1.6 References

[1] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[2] M.A. Abdel-Rahman, Y. Tashiro, K. Sonomoto, Recent advances in lactic acid production by microbial fermentation processes., Biotechnol. Adv. 31 (2013) 877–902. doi:10.1016/j.biotechadv.2013.04.002.

[3] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[4] A.F. Morales, J. Albet, G. Kyuchoukov, G. Malmary, J. Molinier, Influence of Extractant (TBP and TOA), Diluent, and Modifier on Extraction Equilibrium of Monocarboxylic Acids, J. Chem. Eng. Data. 48 (2003) 874–886.

[5] J. Prochazka, A. Heyberger, V. Bizek, M. Kousova, E. Volaufova, Amine Extraction of Hydroxycarboxylic Acids. 2. Comparison of Equilibria for Lactic, Malic, and Citric Acids, Ind. Eng. Chem. Res. 33 (1994) 1565–1573. doi:10.1021/ie00030a016.

[6] J.A. Tamada, A.S. Kertes, C.J. King, Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, Ind. Eng. Chem. Res. 29 (1990) 1319–1326. doi:10.1021/ie00103a035.

[7] B.S. Chanukya, M. Kumar, N.K. Rastogi, Optimization of lactic acid pertraction using liquid emulsion membranes by response surface methodology, Sep. Purif. Technol. 111 (2013) 1–8.

[8] R. Juang, S. Lee, R. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239.

[9] R. Juang, R. Huang, Separation of citric and lactic acids in aqueous solutions by solvent extraction and liquid membrane processes, J. Memb. Sci. 136 (1997) 89–99.

[10] Z. Li, W. Qin, Y. Dai, Liquid-liquid equilibria of acetic, propionic, butyric, and valeric acids with trioctylamine as extractant, J. Chem. Eng. Data. 47 (2002) 843–848.

[11] A. Senol, Liquid−Liquid Equilibria for Mixtures of (Water + Pyruvic Acid + Alcohol / Alamine). Modeling and Optimization of Extraction, J. Chem. Eng. Data. 58 (2013) 528–536. doi:10.1021/je3012265.

[12] Z. Li, W. Qin, Y. Dai, Liquid-liquid equilibria of aqueous acetic acid derivatives with trioctylamine and select organic diluents, J. Chem. Eng. Data. 25 (2003) 1113–1119.

[13] R. Juang, R. Huang, Equilibrium studies on reactive extraction of lactic acid with an amine extractant, Chem. Eng. J. 65 (1997) 47–53.

[14] T. Kirsch, G. Maurer, Distribution of oxalic acid between water and organic solutions of tri-n-octylamine, Ind. Eng. Chem. Res. 35 (1996) 1722–1735.

[15] M.E. Marti, T. Gurkan, L.K. Doraiswamy, Equilibrium and Kinetic Studies on Reactive Extraction of Pyruvic Acid with Trioctylamine in 1-Octanol, Ind. Eng. Chem. Res. 50 (2011) 13518–13525. doi:10.1021/ie200625q.

[16] H. Ziegenfuβ, G. Maurer, Distribution of acetic acid between water and organic solutions of tri-n-octylamine, Fluid Phase Equilib. 102 (1994) 211–255.

[17] T. Kirsch, H. Ziegenfuß, G. Maurer, Distribution of citric, acetic and oxalic acids between water and organic solutions of tri-n-octylamine, Fluid Phase Equilib. 129 (1997) 235–266.

[18] R. Bar, J.L. Gainer, Acid Fermentation in Water-Organic Solvent Two-Liquid Phase Systems, Biotechnol. Prog. 3 (1987) 109–114. doi:10.1002/btpr.5420030208.

[19] F.A. Castillo Martinez, E.M. Balciunas, J.M. Salgado, J.M. Domínguez González, A. Converti, R.P.D.S. Oliveira, Lactic acid properties, applications and production: A review, Trends Food Sci. Technol. 30 (2013) 70–83. doi:10.1016/j.tifs.2012.11.007.

[20] I.M. Mujtaba, E.A. Edreder, M. Emtir, Significant thermal energy reduction in lactic acid production process, Appl. Energy. 89 (2012) 74–80. doi:10.1016/j.apenergy.2010.11.031.

[21] D. Pinelli, F. Magelli, D. Matteuzzi, Production of L (+) and D (-) Lactic Acid Isomers by Lactobacillus casei subsp. casei DSM 20011 and Lactobacillus coryniformis subsp. torquens DSM 20004 in Continuous Fermentation, J. Ferment. Bioeng. 81 (1996) 548–552.

[22] E. Sabolová, Š. Schlosser, J. Marták, Liquid−Liquid Equilibria of Butyric Acid in Water + Solvent Systems with Trioctylamine as Extractant, J. Chem. Eng. Data. 46 (2001) 735–745. doi:10.1021/je000323a.

[23] S.I. Sandler, Chemical, Biochemical, and Engineering Thermodynamics, 4th ed., John Wiley & Sons Inc, Hoboken, NJ, 2006.

[24] M. San-Martín, C. Pazos, J. Coca, Liquid–liquid extraction of lactic acid with Alamine 336, J. Chem. Technol. Biotechnol. 336 (1996) 281–285.

[25] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[26] H. Renon, J.M. Prausnitz, Local compositions in thermodynamic excess functions for liquid mixtures, AIChE J. 14 (1968) 135–144. doi:10.1002/aic.690140124.

[27] D.A. Skoog, D.M. West, F.J. Holler, S.R. Crouch, Fundamentos de Química Analítica, 8th ed., THOMSOM, Paracuellos de Jarama, 2005.

2.2 Liquid-liquid equilibria of lactic acid/water solutions in tri-iso-octylamine/dodecane/1-dodecanol at 306.1, 310.1 and 316.1 K. Experimental data and prediction[2]

Abstract

The liquid-liquid equilibria of systems that involves lactic acid in the aqueous phase and tri-iso-octylamine with diluents as dodecane and 1-dodecanol (active or/and inert) were measured experimentally at three temperatures (306.15, 310.15 and 316.15 K). A previous liquid-liquid equilibrium model that is based on Nernst's distribution law and mass action law equilibrium equations was extended and generalized for stoichiometric ratios (amine:acid) 1:*n*. The effect of the diluents and the tertiary amine on the liquid-liquid equilibrium is shown and quantified in terms of the predicted values of the distribution coefficient, chemical equilibrium constants, and temperature. The lactic acid concentration in equilibrium for the organic phase decreases as follows: water/LA/TiOA/1-dodecanol system > water/LA/TiOA/dodecane/1-dodecanol > system water/LA/TiOA/dodecane system.

[2] This section has been published in: *J. Chem. Eng. Data* 2019, 64, 603−610: Alan D. Pérez, Sneyder Rodríguez-Barona, Javier Fontalvo

2.2.1 Introduction

Liquid-liquid equilibrium of systems where an organic acid splits between an aqueous phase and an organic phase has been widely studied to test new and efficient extractive processes for removal and purification of organic acids [1] to reduce the cost of the final separation steps for the process [2]. Long-chain aliphatic amines have proved to be efficient for organic acid extraction from diluted solutions [3–8], where tertiary amines have been most used and tested in several liquid-liquid equilibria with organic acids such as gallic [9], tartaric [8], butyric [10], and lactic [11] acids, among others.

There are two routes for the transport of the organic acid from the aqueous phase to the organic phase which contains a tertiary amine. Single diffusion of the organic acid and chemical reaction of the tertiary amine with the organic acid to produce an acid-amine complex. This reaction can take place in the liquid interphase or within the organic phase bulk. Moreover, there are two mechanisms for acid-amine complex formation, ion-pair and H-bond [4–6]. The mechanism of ion-pair takes place when the amine is basic enough to bind a proton and form the ammonium cation [4] in order to react with the dissociated organic acid. The H-bond mechanism occurs when the amine is not basic enough to dissociate the extracted organic acid [4]. Thus the amine reacts with the undissociated organic acid. However, the H-bond is possible, when the amine that forms the acid-amine complex through ion-pair is basic enough to bind additional molecules and reacts with the organic acid by the H-bond mechanism as well [4]. In the case of the formation of the acid-amine complex by ion-pair mechanism, the back-extraction process is facilitated because its interaction is weakened when the temperature rises [5].

There are several stoichiometric ratios (amine:acid) that arise in the reaction of an organic acid with a tertiary amine. When the organic acid concentration is low in the organic phase (below stoichiometric ratio to the tertiary amine), the most common stoichiometric ratio of the acid-amine is 1:1 [11–13]. The complexes with stoichiometric ratios of 1:2 and 1:3 are formed at high organic acid concentrations in the organic phase [12–14]. The formation of the stoichiometric ratio 1:2 results from a second organic acid molecule that bonds (H-bond) to the organic acid that is already in the 1:1 form [12]. Therefore, the stoichiometric ratio 1:3 comes from a third organic acid molecule that interacts with the complex that is in the 1:2 form through H-bond [12]. Generally, both mechanisms, ion-pair and H-bond, are involved in the extraction process by tertiary amines [6].

The tertiary amines used for organic acid extraction are generally combined with some diluents in order to improve the physical properties of the organic phase such as density, viscosity, interfacial tension, and the extractive capacity [7–9]. Most of these diluents can be classified as inert or active diluents [15]. Inert diluents are usually nonpolar organic compounds [16] that are used to improve the physical properties of the organic phase [15]. Active diluents (called modifiers [15] as well) are organic compounds that contain polar groups with the capacity of stabilizing the bond of the formed acid-amine complex by solvation [16,17] favoring the extraction process [17]. Active diluents provide a strong effect on the extraction mechanism [5] and change the activity coefficients of the complex [12]. For instance, the acid and the amine form a complex by ion-pair in an organic phase containing an active diluent while it forms a complex by H-bond in an organic phase with an inert diluent [16]. For the case of the lactic acid (LA) liquid-liquid equilibrium (LLE), the temperature effect depends on the kind of diluent and the extractant that is used [12].

Tertiary amines such as trioctylamine (TOA) and tri-iso-octylamine (TiOA) have been often used for LA removal as extractants [11,13,15,18–20]. On the other hand, diluents such as dodecane [21,22], dodecanol [11], and oleyl alcohol [6,23] have been used for removal of several organic acids.

In this work, the LLE for systems water/LA/tri-iso-octylamine/dodecane, water/LA/tri-iso-octylamine/1-dodecanol, and water/LA/tri-iso-octylamine/dodecane/1-dodecanol have been tested experimentally at three temperatures (306.15, 310.15 and 316.15 K), where usually LA fermentation is carried out [24–26]. Also, in several studies of liquid-liquid extraction and liquid membranes, there is no significant enhanced of LA removal at TOA concentration higher than 30 vol%(around 30 and 35 mol%) [12,18,27]. On the other hand, focused on an in-situ removal application, tertiary amines, such as TOA has shown a toxic effect on specific strains of *Lactobacillus casei* bacteria [28]. Additionally, we carried out, toxicity studies of the TiOA, dodecane, dodecanol, among others, on *Lactobacillus casei* ATCC 393 and it shows that toxicity of TiOA on this specific microorganisms (which produces LA) increases as TOA or TiOA concentration rises. However, for low concentrations of TiOA (around 22 mol%) there is a low toxic effect on the specific microorganism. This study was carried out toward in its application for LA removal from fermentation broths using reactive extraction or liquid membranes. From this point of view, the LA concentrations are around of 20 $g \cdot L^{-1}$ for a conventional batch fermentation [24–26], and even higher LA concentrations can be achieved using modified microorganisms and optimizing the operating conditions [24–26,29].

Additionally, a previous liquid-liquid equilibrium model, based on Nernst's distribution law and mass action law equilibria equations [11], has been extended to generalized stoichiometric ratios 1:*n* in order to predict the values of the distribution coefficient and equilibrium constants.

2.2.2 Experimental section

Materials

Tri-iso-octylamine, n-dodecane and 1-dodecanol have a low solubility in water, lower than 1 $g \cdot L^{-1}$ at 293.15 K, practically insoluble at 298.15 K, and 0.004 $g \cdot L^{-1}$ at 293.15 K, respectively. The purity of lactic acid was assessed by titration with sodium hydroxide using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). A stock solution of lactic acid (150 $g \cdot L^{-1}$) was heated at 363.1 K under total reflux between 8 and10 h for dimer hydrolysis [30,31] and subsequently, the lactic acid concentration was measured by titration. Type I water was used for all aqueous solutions (Barnstead™ Nanopure™). All chemicals used are listed in Table 1.

Table 1. Chemicals used for the experiments. Physicochemical properties were taken from their respective MSDS of the supplier (each one at room temperature).

Name	CAS	Supplier	Molecular weight	Density ($g \cdot mL^{-1}$)	Purity (wt%)
Tri-iso-octylamine	25549-16-0	Merck Millipore	353.68	0.8	95
n-dodecane	112-40-3	Merck Millipore	170.34	0.75	99
1-dodecanol	112-53-8	Merck Millipore	186.33	0.83	98
L(+)-lactic acid	79-33-4	Panreac Química S.A.U.	90.08	1.206	88-92
Sodium hydroxide	1310-73-2	Carlo Erba	39.997	2.13	97

Experimental Procedure and Analysis.

Three organic phases were tested. All organic phases contained tri-iso-octylamine (TiOA) at 5.37 mol% (corresponding to 10 vol%) as the extractant, taking into account organic acid removal capacity and toxicity of the amine on specific probiotic lactic acid bacteria. The three organic phases contained as diluents 1-dodecanol and dodecane at 42.33 mol% (40 vol%) and at 52.30 mol% (50 vol%), respectively. The aforementioned organic phases were placed in contact with seven aqueous

solutions of lactic acid at concentrations from 0.02 to 0.73 mol% (corresponding to 1 to 37 $g \cdot L^{-1}$), where usually LA fermentations are carried out [24–26]. For the liquid-liquid equilibria (LLE) experiments and each organic phase, 3 mL of the organic solution and 3 mL of the aqueous solution were added into a vial of 10 mL. The experimental procedure was carried out at three temperatures (306.15, 310.15, and 316.15 K) following the subsequent steps: agitation, decantation, and sampling. First, all vials were shaken at 180 rpm (Wiseshake Shot-1D, Wisd) within an incubator (Wisd, ± 0.6 K) for 72 h. Afterward, the agitation was stopped and the vials were kept in the incubator (decantation step) for 72 h. One sample of the aqueous solution was taken from every single vial and its lactic acid concentration was measured by high performance liquid chromatography, HPLC (Elite LaChrom). For HPLC analysis, ORH-801 column (Chrom Tech) with a solution of 0.01 N of sulfuric acid (Merck, assay 95-97%) for the mobile phase, and a RI detector at 318.15 K it was used. The lactic acid concentration in the organic phase was obtained by back-extraction putting 1 mL of each organic phase (in equilibrium) in contact with 3 mL of an aqueous solution of sodium hydroxide (Merck, assay 99%) at 20 $g \cdot L^{-1}$ during 25 h (1 h of agitation and 24 h of decantation at 310.1 K). Afterward, the lactic acid concentration in the resulting aqueous phase was measured by HPLC. Each LLE experiment was carried out in triplicate.

Additionally, the above procedure was also performed for organic phases containing only the diluent. This is dodecane for the first system, 1-dodecanol for the second system, and the mixture dodecane with 1-dodecanol at 59.72 and 40.28 mol% (60 and 40 vol%), respectively for the third system. Three concentrations of the aqueous phase were used from 1 to 37 $g \cdot L^{-1}$ of lactic acid, due to an expected linear trend of the data because there is only a physical extraction (in absence of the amine) which is represented for the Nernst's distribution law (which is a linear equation).

A sample of the organic phase in equilibrium (corresponding to the initial concentration of LA in the aqueous phase of 1 and 37 $g \cdot L^{-1}$) was taken in order to measure the amount of water in the organic phase through Karl Fischer titration using a Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). All LLE experiments were carried out at atmospheric pressure (77.9 kPa).

2.2.3 Theoretical section

This model involves the distribution of the lactic acid (or any organic acid) in the aqueous phase and the organic phase as follows:

$$LA_{aq} \overset{K_D}{\rightarrow} LA_{org} \tag{1}$$

$$K_D = \frac{[LA]_{org}}{[LA]_{aq}} \tag{2}$$

The distribution coefficient (eq 2) includes the concentrations of free lactic acid (LA) in equilibrium in both phases. However, in the organic phase there is also LA in its complex form as shown below (the brackets represents molar concentrations).

The chemical reaction in the organic phase occurs between free LA and TiOA in order to produce a LA-TiOA complex given by the following reaction (eq 3):

$$nLA_{org} + mTiOA_{org} \overset{K_E}{\leftrightarrow} \left((LA)_n (TiOA)_m\right)_{org} \tag{3}$$

For monoprotic acids, only one molecule of the tertiary amine is bonded to one or more molecules of the organic acid. Therefore, the stoichiometric of the case above can be assumed as 1:*n*.

This work is focused on the development of a general model for stoichiometric ratios 1:*n*. However, to get a general model, first the model for stoichiometric ratio 1:1, developed in a previous work [11] is shown below:

$$K_D [LA]_{aq} \left([TiOA]^0_{org} + K_D [LA]_{aq} - [LA]_{org,Tot}\right) \cdot \left(K_{E,1:1}\right) = [LA]_{org,Tot} - K_D [LA]_{aq} \tag{4}$$

In eq 4, the total concentration of LA in the organic phase is a function of the LA concentration in the aqueous phase as was shown in a previous work [11].

As was mentioned above, the formation of the complex of stoichiometric ratio 1:2 requires the formation of the complex of stoichiometric ratio 1:1 (eq 5). Therefore, the development of the model for the stoichiometric ratio the 1:2 involves the following equations:

$$K_{E,1:1} = \frac{[(LA)(TiOA)]_{org}}{[LA]_{org}[TiOA]_{org}} \tag{5}$$

$$K_{E,1:2} = \frac{[(LA)_2(TiOA)]_{org}}{[LA]^2_{org}[TiOA]_{org}} \tag{6}$$

$$[LA]_{org,Tot} = [LA]_{org} + [(LA)(TiOA)]_{org} + [(LA)_2(TiOA)]_{org} \tag{7}$$

$$[TiOA]^0_{org} = [TiOA]_{org} + [(LA)(TiOA)]_{org} + [(LA)_2(TiOA)]_{org} \tag{8}$$

Combining eqs 2, 5-8 the following expression for the LLE model can be obtained:

$$K_D[LA]_{aq}\left([TiOA]^0_{org} + K_D[LA]_{aq} - [LA]_{org,Tot}\right)\cdot\left(K_{E,1:1} + K_{E,1:2}K_D[LA]_{aq}\right) = [LA]_{org,Tot} - K_D[LA]_{aq} \quad (9)$$

In the same way for the stoichiometric ratio 1:3, the equilibrium constants (1:1, 1:2 and 1:3), the mass balance for LA in the organic phase and TiOA mass balance are taking into account to achieve the following equation:

$$K_D[LA]_{aq}\left([TiOA]^0_{org} + K_D[LA]_{aq} - [LA]_{org,Tot}\right)\cdot \left(K_{E,1:1} + K_{E,1:2}K_D[LA]_{aq} + K_{E,1:3}K_D^2[LA]^2_{aq}\right) = [LA]_{org,Tot} - K_D[LA]_{aq} \quad (10)$$

Based on eqs 4, 9, 10 is possible to generalize an equation for a ratio (1:*n*) to obtain (where *N* is the higher considered stoichiometric coefficient for the organic acid):

$$K_D[LA]_{aq}\left([TiOA]^0_{org} + K_D[LA]_{aq} - [LA]_{org,Tot}\right)\cdot\sum_{n=1}^{N}\left\{K_{E,1:n}K_D^{n-1}[LA]^{n-1}_{aq}\right\} = [LA]_{org,Tot} - K_D[LA]_{aq} \quad (11)$$

The values of $K_{E,1:n}$ and K_D were calculated at each temperature by minimizing the sum of squares of the deviations between experimental and predicted LA concentrations in the organic phase using *globalsearch* function of Matlab® as follows:

$$f_{obj} = \sum_{j=1}^{z}\left([LA]^{pred}_{org} - [LA]^{\exp}_{org}\right)^2 \quad (12)$$

where, *z* is the amount of experimental data and the superscripts *pred* and *exp*, are the predicted and experimental data, respectively.

Enthalpy and entropy change on reaction were calculated based on the Van't Hoff equation in the range of temperatures of 306.1 to 316.1 K as it is shown somewhere else [11].

2.2.4 Results and discussion

Three liquid-liquid equilibria systems were experimentally tested with and without adding the tertiary amine (TiOA). Their distribution coefficient and chemical equilibrium constant were fitted using the proposed model in this work as it is shown below.

Systems water/LA/dodecane and water/LA/TiOA/dodecane

Experimental results of the liquid-liquid equilibrium of the system water/LA/dodecane showed low values of the equilibrium lactic acid (LA) concentration in the organic phase. For the lowest temperature tested (306.15 K), it was not possible to measure the equilibrium LA concentration in the organic phase. Since LA has a low bulk solubility on dodecane and also, dodecane does not promote solvation of the TiOA complex, the LA solubility in dodecane is reduced at low temperatures. For temperatures of 310.15 and 316.15 K, the equilibrium concentrations of LA in the organic phase were around 1.1×10^{-4} and 3.9×10^{-3} mol·L^{-1} with standard deviations of the same order of magnitude in the same range of LA concentrations. This occurs because these LA concentrations (of the organic phase) were around the limit of quantification in the HPLC.

Table 2. Distribution coefficients (K_D) and correlation coefficients (R^2) obtained by linear fit for the water/LA/dodecane/LA for the three temperatures (306.1, 310.1, and 316.1 K).

Temperature (K)	K_D	R^2
306.1	-	-
310.1	0.0049	0.916
316.1	0.0093	0.973

The distribution coefficients (eq 2) for LA in the organic phase that only contains dodecane were calculated from experimental data at three temperatures (306.15, 310.15, and 316.15 K) and are shown in Table 2. However, at the lower temperature, it was not possible to measure due to the low LA concentrations in the organic phase. The results show that the solubility of LA in dodecane is low and the higher is the temperature, the higher is the distribution coefficient (Table 2). The low values of the R^2, especially at 316.15 K was due to the high standard deviations of the experimental measurements of LA concentrations.

Table 3. Distribution coefficients (K_D), equilibrium constants (K_{eq}) and correlation coefficients (R^2) using the proposed ELL model for the water/LA/TiOA/dodecane system for the three temperatures (306.1, 310.1, and 316.1 K).

Temperature (K)	Linear fit		Proposed LLE model		
	K_D	R^2	K_D	Log($K_{eq,1:1}$)	R^2
306.1	0.0156	0.938	0.0156	-7.23	0.938
310.1	0.0194	0.894	0.0194	-7.14	0.894
316.1	0.0204	0.945	0.0204	-7.14	0.945

The experimental LLE for the system water/LA/tri-iso-octylamine (TiOA)/dodecane is shown in Figure 1 at the three temperatures. This system also shows a low solubility (of the LA into organic phase) and similar influence of temperature on the LLE as for the system without TiOA. The equilibrium LA concentration in the organic phase for the system without TiOA was around 21.4 and 50% of the corresponding values for the system with TiOA at 310.1 and 316.1 K, respectively (measured at the maximum value of LA concentration in equilibrium). In Table 3, the calculated values of the distribution coefficients are shown, which were fitted through a linear fit and using the proposed model of this work with a stoichiometric ratio of 1:1. The fitted values were the same for both, the proposed model and the linear fitting. These results are the same only when the value of the chemical equilibrium constants are low enough to be neglected. For a low value of the chemical equilibrium constant, eq 11 (for a ratio 1:1) is reduced to eq 2. The equilibrium constant at the three evaluated temperatures is around 7×10^{-4} for this system.

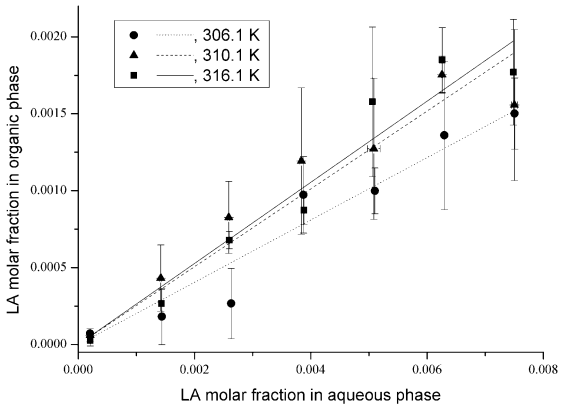


Figure 1. Experimental data (symbols) and fitted model (lines) of the liquid-liquid equilibria for the water/LA/TiOA/dodecane system at three temperatures (306.1, 310.1, and 316.1 K).

According to the experimental results of the system with only dodecane in the organic phase, the dodecane provides a low distribution of LA in the system. However, the LA concentration in the organic phase was slightly increased by adding the TiOA. The TiOA can react with the LA in the interphase and within the organic phase bulk, but the complex that is produced between the amine and the LA is not stabilized by solvation of the diluent (dodecane). TiOA can solvate the LA in the organic phase by its basicity [32], therefore, the amount of LA in the organic phase in equilibrium slightly increases.

The values of the R^2 for both systems (with and without TiOA) were between 0.89 and 0.97. These relatively low values of R^2 are due to the experimental LA concentrations were around of the limit of quantification in the HPLC, specifically in the system without the amine where the LA concentrations in the organic phase were significantly low.

Systems water/LA/1-dodecanol and water/LA/TiOA/1-dodecanol

The distribution coefficient (eq 2) for the system water/LA/1-dodecanol, slightly increases, from 0.1020 to 0.1117, as the temperature rises, from 306.1 to 316.1 K, respectively (Table 4), the experimental data of which are shown in Figure 2. As it is expected, the solubility increases as temperature rises. These distribution coefficients (system without TiOA and with 1-dodecanol) were 2 orders of magnitude higher than the distribution coefficients of the system with dodecane (water/LA/dodecane and water/LA/TiOA/dodecane). This occurs because 1-dodecanol provides of a solvation shell to the LA that is solubilized into the organic phase [32].

Table 4. Distribution coefficients (K_D) and correlation coefficients (R^2) obtained by linear fit for the water/LA/1-dodecanol system for the three temperatures (306.1, 310.1, and 316.1 K).

Temperature (K)	K_D	R^2
306.1	0.1021	0.998
310.1	0.1059	0.990
316.1	0.1117	0.992

For the water/LA/TiOA/1-dodecanol system (Table 5 and Figure 3), also the distribution coefficient increases as the temperature rises. However, TiOA has an important positive effect on the LA solubility in the organic phase. For instance, for a LA concentration of 0.2 $mol \cdot L^{-1}$ in the aqueous phase, the LA concentration in the organic phase within 1-dodecanol increases 1 order of magnitude from around 0.025 (without TiOA) to 0.20 $mol \cdot L^{-1}$ (with TiOA). It is because both 1-dodecanol and

TiOA can solvate the free LA in the organic phase and 1-dodecanol solvates the LA-TiOA complex stabilizing it.

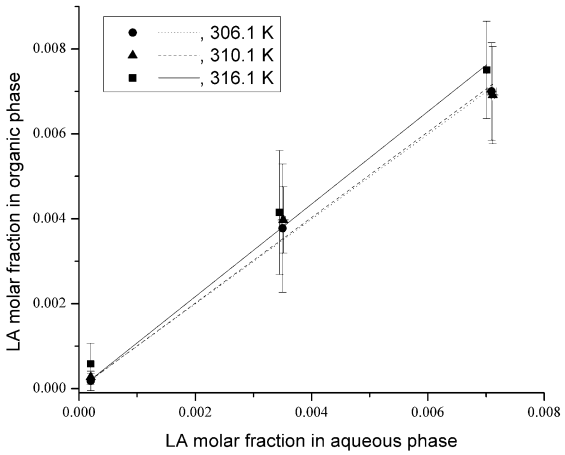


Figure 2. Experimental data (symbols) and fitted model (lines) of the liquid-liquid equilibria for the water/LA/1-dodecanol system at three temperatures (306.1, 310.1, and 316.1 K).

In the system including TiOA (Figure 3), the chemical equilibrium constants $K_{E,1:1}$ and $K_{E,1:2}$ were calculated. The chemical equilibrium constant $K_{E,1:2}$ was low enough to be neglected (with an order of magnitude of 1×10^{-3}) at the three temperatures. Therefore, the 1:1 stoichiometric ratio can be assumed for this system.

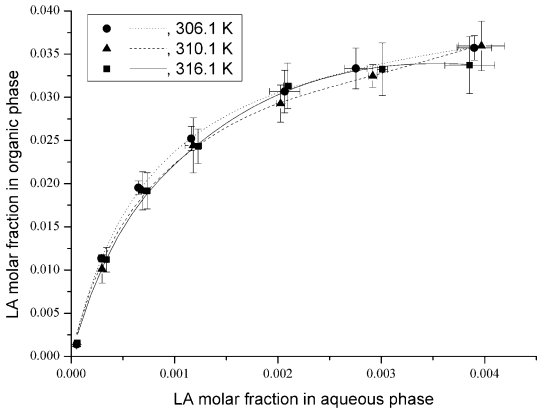


Figure 3. Experimental data (symbols) and fitted model (lines) of the liquid-liquid equilibria for the water/LA/TiOA/1-dodecanol system at three temperatures (306.1, 310.1, and 316.1 K).

On the other hand, the chemical equilibrium constant ($K_{E,1:1}$) decreases as the temperature rises (Table 5), suggesting an exothermic reaction (ΔH_{rxn} = -24.41 kcal·mol^{-1} and ΔS_{rxn} = -0.0677 kcal·(mol·K)$^{-1}$ calculated using Van't Hoff equation[11]). The values of R^2 in the fit for the proposed model of this system (with and without TiOA) were around 0.99 at the three temperatures.

Table 5. Distribution coefficients (K_D), equilibrium constants (K_{eq}) and correlation coefficients (R^2) using the proposed ELL model for the water/LA/TiOA/1-dodecanol system for the three temperatures (306.1, 310.1, and 316.1 K).

Temperature (K)	K_D	Log($K_{eq,1:1}$)	Log($K_{eq,1:2}$)	R^2
306.1	0.0489	2.64	-2.31	0.998
310.1	0.0893	2.38	-2.30	0.997
316.1	0.1476	2.08	-2.39	0.998

Systems water/LA/dodecane/1-dodecanol and water/LA/TiOA/dodecane/1-dodecanol.

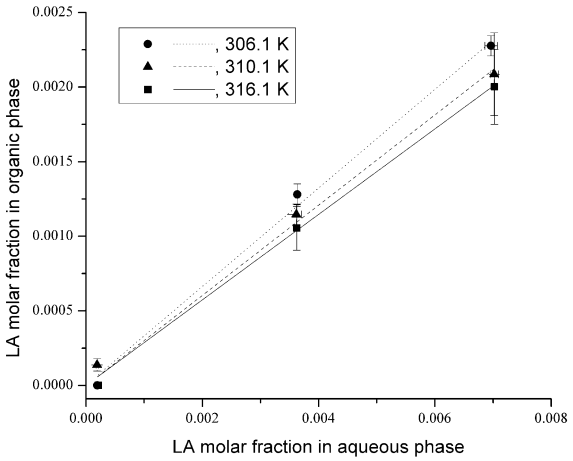


Figure 4. Experimental data (symbols) and fitted model (lines) of the liquid-liquid equilibria for the water/LA/dodecane/1-dodecanol system at three temperatures (306.1, 310.1, and 316.1 K).

Figure 4 shows measured LLE for this system without the amine (at 39.72 mol% of 1-dodecanol in dodecane). In this case, the distribution coefficient slightly decreases as temperature rises (Table 6). However, this decrease is within the experimental standard deviation (from 0.029 to 0.024 at 306.1 and 316.1 K, respectively), hence, it can be considered that there is not an appreciable effect of the temperature on the distribution coefficient, which is around of 0.02 at the three temperatures.

Table 6. Distribution coefficients (K_D) and correlation coefficients (R^2) obtained by linear fit for the water/LA/dodecane/1-dodecanol system for the three temperatures (306.1, 310.1 and 316.1 K).

Temperature (K)	K_D	R^2
306.1	0.0290	0.997
310.1	0.0259	0.994
316.1	0.0244	0.998

In the LLE for the water/LA/TiOA/dodecane/1-dodecanol system (Figure 5) the equilibrium LA concentration in the organic phase decreases as temperature rises (Table 7). The equilibrium LA concentration of the organic phase for this system with TiOA decreased 40% compared to the system of water/LA/TiOA/1-dodecanol.

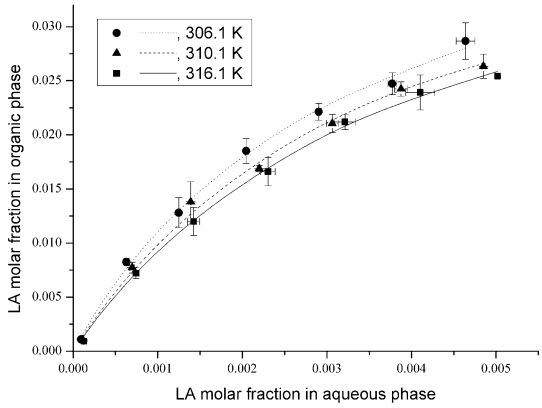


Figure 5. Experimental data (symbols) and fitted model (lines) of the liquid-liquid equilibria for the water/LA/TiOA/dodecane/1-dodecanol system at three temperatures (306.1, 310.1 and 316.1 K).

Table 7. Distribution coefficients (K_D), equilibrium constants (K_{eq}) and correlation coefficients (R^2) using the proposed ELL model for the water/LA/TiOA/dodecane/1-dodecanol system (306.1, 310.1 and 316.1 K).

Temperature (K)	K_D	Log($K_{eq,1:1}$)	Log($K_{eq,1:2}$)	R^2
306.1	0.0409	2.09	-2.30	0.997
310.1	0.0313	2.14	-1.42	0.996
316.1	0.0212	2.27	-2.30	0.998

The values of the chemical equilibrium constants $K_{E,1:2}$ were low enough to be neglected in this system of water/LA/TiOA/dodecane/1-dodecanol (Table 7). An exothermic reaction is expected; however, according to the values of the chemical equilibrium $K_{E,1:1}$, the reaction adopted a slightly endothermic behavior (ΔH_{rxn} = 8.34 kcal·mol^{-1} and ΔS_{rxn} = -0.0368 kcal·(mol·K)$^{-1}$ calculated using Van't Hoff equation [11]). It is unclear why the chemical equilibrium constant decreases as the temperature slightly rises. It requires additional studies to determine the structure of the nuclei aggregates, the solvation, and the competitive complexation mechanisms of the involved substances (water/LA/TiOA/LA-TiOA/dodecane/1-dodecanol). In the fit of this system, the values of R^2 were around 0.99.

Water co-extraction

The water concentration in the organic phase was measured for the three aforementioned systems and it is shown in Table 8. For the system water/LA/dodecane, the water amount in the organic phase was low and it stayed practically constant with temperature and concentration of LA, around of 0.15 wt%. The same behavior is shown for this system with TiOA, the water concentration being around 0.29 wt%. The water concentration in the first system was low, always lower than 1 wt% both with TiOA and without TiOA; however, the water concentration in the organic phase increased almost twice with TiOA.

In the system water/LA/1-dodecanol the maximum water concentration was 3.94 wt% at 316.1 K, which corresponds to the highest LA concentration in the aqueous phase. The higher the temperature and the LA concentration in the aqueous phase are, the higher the water concentration in the organic phase will be. The water concentration is reduced to 3.03 wt% by the presence of TiOA (5.37 mol%). Water has a higher solubility in 1-dodecanol than its solubility in the TiOA/dodecane mixture.

On the other hand, it was observed in the above LLE results that the system water/LA/TiOA/1-dodecanol provides the highest equilibrium concentration of LA in the organic phase. The presence of water in the organic phase supports this effect. Molecules of water within the organic phase can interact with the extractant (TiOA) and the solute (LA) forming several nuclei aggregates. Water provides H-bonding for solvation and even can compete with the active diluent (1-dodecanol) for the formation of the solvation shell [32]. Therefore, the higher the amount of water in the organic phase, the higher the solvation effect of the LA and LA-TiOA complex in the organic phase.

Table 8. Water concentration in the organic phase for the liquid-liquid equilibria of the three systems at the temperatures of 306.1, 310.1 and 316.1 K and at 77.9 kPa[a] at 0.2 mol% (1 g·L^{-1}) and at 0.73 mol% (37 g·L^{-1}) aqueous phase.

Substances of the organic phase	Mol%	T/K	w^{org} (wt%)	
			at the lowest LA concentration (0.2 mol%)	at the highest LA concentration (0.73 mol%)
Dodecane[b]	100	306.1	0.13	0.15
		310.1	0.15	0.16
		316.1	0.15	0.15
Dodecane/TiOA[c]	94.63/5.37	306.1	0.27	0.30
		310.1	0.29	0.31
		316.1	0.26	0.30
1-dodecanol[d]	100	306.1	2.91	3.32
		310.1	3.22	3.70
		316.1	3.60	3.94
1-dodecanol/TiOA[e]	94.63/5.37	306.1	1.48	2.75
		310.1	2.47	3.05
		316.1	2.39	3.03
Dodecane/1-dodecanol[f]	39.73/60.27	306.1	0.78	1.06
		310.1	0.81	0.95
		316.1	0.73	0.73
Dodecane/1-dodecanol/TiOA[g]	52.85/41.79/5.37	306.1	0.79	1.32
		310.1	0.81	1.19
		316.1	0.73	1.16

[a] Standard uncertainty $u(T)$ = 0.6 K and $u(P)$ = 0.075 kPa. [b] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.3 and at the highest LA concentration $u_r(w_{org})$ = 0.2. [c] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.3 and at the lowest LA concentration $u_r(w_{org})$ = 0.1. [d] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.04 and at the lowest LA concentration $u_r(w_{org})$ = 0.01. [e] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.1 and at the lowest LA concentration $u_r(w_{org})$ = 0.05. [f] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.1 and at the lowest LA concentration $u_r(w_{org})$ = 0.1. [g] Standard uncertainties at the lowest LA concentration $u_r(w_{org})$ = 0.05 and at the lowest LA concentration $u_r(w_{org})$ = 0.04.

In the systems water/LA/dodecane/1-dodecanol and water/LA/TiOA/dodecane/1-dodecanol, the higher the LA concentration in the organic phase and the lower the temperature are, the higher is the water concentration in the organic phase. The fact that the water concentration decreases as temperature rises in this system can provide an explanation of why the distribution coefficients decrease as temperature rises in the system with TiOA. In this system, water and 1-dodecanol can

solvate the LA and LA-TiOA, therefore the availability of solvating molecules is reduced as water concentration decreases.

2.2.5 Conclusions

The liquid-liquid equilibria of the systems, water/lactic acid/dodecane/tri-iso-octylamine, water/lactic acid/1-dodecanol/tri-iso-octylamine, and water/lactic acid/dodecane/1-dodecanol/tri-iso-octylamine, including the respective systems without the tertiary amine, were tested experimentally at three temperatures (306.1, 310.1, and 316.1 K).

The concentration of lactic acid (LA) in equilibrium is higher in the systems with TiOA compared with systems without it. LA concentration is also favored as temperature rises for the systems water/lactic acid/dodecane/tri-iso-octylamine and water/lactic acid/1-dodecanol/tri-iso-octylamine. However, for the system water/lactic acid/dodecane/1-dodecanol/tri-iso-octylamine the opposite behavior was observed with the increase of the temperature: the higher was the temperature, the lower was the LA equilibrium concentration in the organic phase.

The 1-dodecanol provides higher solubility of water compared with the system with only dodecane and the other ones that include the tertiary amine.

The proposed liquid-liquid equilibrium model is an extension of a previous model (from stoichiometric ratios 1:1 to 1:*n*) based on Nernst's distribution law and mass action law equilibria equations. This model can be used for systems in which an organic acid is involved in the aqueous phase and a tertiary amine within one or several diluents is involved in the organic phase. Also, it can be used for systems where the acid-amine complex is formed for several stoichiometric ratios. This model is in agreement with the experimental values of the liquid-liquid equilibria for the tested systems providing the stoichiometric ratio between the tertiary amine and the LA, the distribution coefficient and the chemical equilibrium constant.

The experimental liquid-liquid equilibria and its predictive model are useful for the design of liquid extraction and liquid membrane and its intensified processes for LA removal, for instance, from fermentation broths.

NOTATION

K_D	Distribution coefficient
K_E	Chemical equilibrium constant $[L \cdot mol^{-1}]^n$

LA	Lactic acid
LA-TiOA	The complex between lactic acid and tri-iso-octylamine
m	The stoichiometric coefficient for the TiOA
n	The stoichiometric coefficient for the LA
N	The highest stoichiometric coefficient for the LA
TiOA	Tri-iso-octylamine (tertiary amine)
w	Mass percentage of water in the organic phase
x	Equilibrium molar fraction
z	Number of experimental data
ΔH_{rxn}	Enthalpy change on reaction [kcal/mol]
ΔS_{rxn}	Entropy change on reaction [kcal/mol·K]

Subscripts and superscripts

0	Initial concentration
aq	Aqueous phase
exp	Experimental data
j	*j*-th data
org	Organic phase
pred	Predicted
Tot	Total

2.2.6 References

[1] N. Tik, E. Bayraktar, Ül. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, J. Chem. Technol. Biotechnol. 76 (2001) 764–768.

[2] S.T. Yang, C. Lu, Extraction-Fermentation Hybrid (Extractive Fermentation), in: Sep. Purif. Technol. Biorefineries, 1st ed., John Wiley & Sons, 2013: pp. 409–437.

[3] S.T. Yang, S.A. White, S.T. Hsu, Extraction of Carboxylic Acids with Tertiary and Quaternary Amines: Effect of pH, Ind. Eng. Chem. Res. 30 (1991) 1335–1342.

[4] A.M. Eyal, R. Canari, pH Dependence of Carboxylic and Mineral Acid Extraction by Amine-Based Extractants: Effects of pKa, Amine Basicity, and Diluent Properties, Ind. Eng. Chem. Res. 34 (1995) 1789–1798.

[5] R. Canari, A.M. Eyal, Extraction of carboxylic acids by amine-based extractants: Apparent extractant basicity according to the pH of half-neutralization, Ind. Eng. Chem. Res. 42 (2003) 1285–1292.

[6] Z. Gu, B.A. Glatz, C.E. Glatz, Propionic acid production by extractive fermentation. I. Solvent considerations, Biotechnol. Bioeng. 57 (1998) 454–461. doi:10.1002/(SICI)1097-0290(19980220)57:4<454::AID-BIT9>3.0.CO;2-L.

[7] H. Ziegenfuß, G. Maurer, Distribution of acetic acid between water and organic solutions of tri-n-octylamine, Fluid Phase Equilib. 102 (1994) 211–255.

[8] M. Marinova, J. Albet, J. Molinier, G. Kyuchoukov, Specific influence of the modifier (1-Decanol) on the extraction of tartaric acid by different extractants, Ind. Eng. Chem. Res. 44 (2005) 6534–6538.

[9] S. Pandey, S. Kumar, Reactive Extraction of Gallic Acid Using Aminic and Phosphoric Extractants Dissolved in Different Diluents: Effect of Solvent's Polarity and Column Design, Ind. Eng. Chem. Res. 57 (2018) 2976–2987.

[10] E. Sabolová, Š. Schlosser, J. Marták, Liquid−liquid equilibria of butyric acid in water + solvent systems with trioctylamine as extractant, J. Chem. Eng. Data. 46 (2001) 735–745.

[11] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria for Trioctylamine/1-Dodecanol/Lactic Acid/Water System at 306.1, 310.1 and 316.1 K: Experimental Data and Prediction, J. Chem. Eng. Data. 61 (2016) 2269–2276.

[12] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[13] W. Qin, Z. Li, Y. Dai, Extraction of Monocarboxylic Acids with Trioctylamine: Equilibria and Correlation of Apparent Reactive Equilibrium Constant, Ind. Eng. Chem. Res. 42 (2003) 6196–6204.

[14] J.A. Tamada, C.J. King, Extraction of carboxylic acids with amine extractants. 2. Chemical Interactions and Interpretation of Data, Ind. Eng. Chem. Res. 29 (1990) 1327–1333.

[15] G. Kyuchoukov, A. Labbaci, J. Albet, J. Molinier, Simultaneous Influence of Active and "Inert" Diluents on the Extraction of Lactic Acid by Means of Tri- n -octylamine (TOA) and Tri- iso -octylamine (TIOA), Ind. Eng. Chem. Res. 45 (2006) 503–510. doi:10.1021/ie050912f.

[16] J. Marták, L. Kubišová, Š. Schlosser, Liquid-liquid equilibria of 5-methyl-2-pyrazinecarboxylic and sulfuric acids for solvents with trioctylamine, J. Chem. Eng. Data. 55 (2010) 3578–3589.

[17] B. Choudhury, T. Swaminathan, Lactic acid extraction with trioctyl amine, Bioprocess Eng. 19 (1998) 317. doi:10.1007/s004490050526.

[18] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[19] D.H. Han, W.H. Hong, Water-Enhanced Solubilities of Lactic Acid in Reactive Extraction Using Trioctylamine/Various Active Diluents Systems, Sep. Sci. Technol. 33 (1998) 271–281. doi:10.1080/01496399808544768.

[20] G. Malmary, J. Albet, A. Putranto, H. Hanine, J. Molinier, Measurement of partition coefficients of carboxylic acids between water and triisooctylamine dissolved in various diluents, J. Chem. Eng. Data. 43 (1998) 849–851. doi:10.1021/je980087s.

[21] A. Labbaci, G. Kyuchoukov, J. Albet, J. Molinier, Detailed investigation of lactic acid extraction with tributylphosphate dissolved in dodecane, J. Chem. Eng. Data. 55 (2010) 228–233. doi:10.1021/je900315r.

[22] A.F. Morales, J. Albet, G. Kyuchoukov, G. Malmary, J. Molinier, Influence of Extractant (TBP and TOA), Diluent, and Modifier on Extraction Equilibrium of Monocarboxylic Acids, J. Chem. Eng. Data. 48 (2003) 874–886.

[23] H. Honda, Y. Toyama, H. Takahashi, Effective lactic acid production by two-stage extractive fermentation, J. Ferment. Bioeng. 79 (1995) 589–593.

[24] M.A. Abdel-Rahman, Y. Tashiro, K. Sonomoto, Recent advances in lactic acid production by microbial fermentation processes., Biotechnol. Adv. 31 (2013) 877–902. doi:10.1016/j.biotechadv.2013.04.002.

[25] F.A. Castillo Martinez, E.M. Balciunas, J.M. Salgado, J.M. Domínguez González, A. Converti, R.P.D.S. Oliveira, Lactic acid properties, applications and production: A review, Trends Food Sci. Technol. 30 (2013) 70–83. doi:10.1016/j.tifs.2012.11.007.

[26] D. Pinelli, F. Magelli, D. Matteuzzi, Production of L (+) and D (-) Lactic Acid Isomers by Lactobacillus casei subsp. casei DSM 20011 and Lactobacillus coryniformis subsp. torquens DSM 20004 in Continuous Fermentation, J. Ferment. Bioeng. 81 (1996) 548–552.

[27] R. Juang, S. Lee, R. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239.

[28] N.A. Marinova, D.S. Yankov, Toxicity of some solvents and extractants towards Lactobacillus casei cells, Bulg. Chem. Commun. 41 (2009) 368–373.

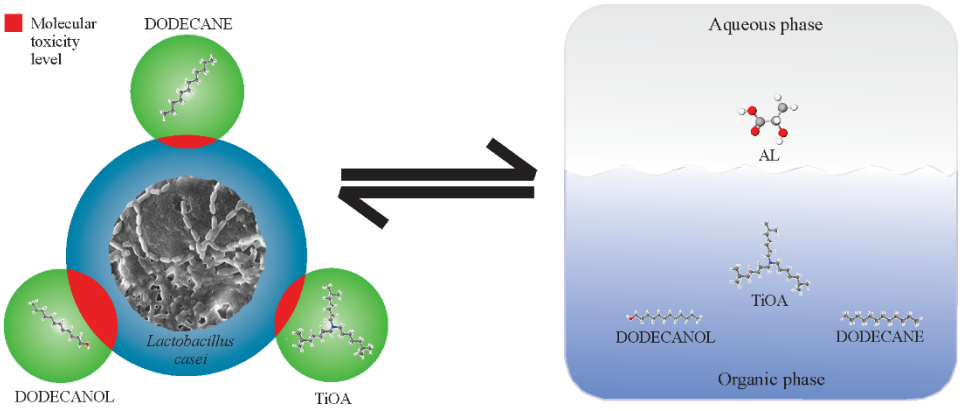
[29] G.B. Brinques, M. Do Carmo Peralba, M.A.Z. Ayub, Optimization of probiotic and lactic acid production by Lactobacillus plantarum in submerged bioreactor systems, J. Ind. Microbiol. Biotechnol. 37 (2010) 205–212. doi:10.1007/s10295-009-0665-1.

[30] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[31] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71. doi:10.1016/j.bej.2004.03.006.

[32] V.S. Kislik, Solvent Extraction: Classical and Novel Approaches, 1st ed., Amsterdam, 2012.

3. Chapter 3: Selection of a membrane phase for in-situ lactic acid removal



3.1 Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using *Lactobacillus casei* ATCC 393[3]

Abstract

Toxic effects of extractants and carriers of specific microorganisms must be taken into account before using them with hybrid fermentation processes that are combined with liquid membranes or liquid-liquid extraction. In the current research three extractants (trioctylamine, tri-iso-octylamine and Aliquat 336), three diluents (dodecane, dodecanol, and oleyl alcohol) and two mixtures (extractant/diluent) were tested for molecular toxicity on the bacteria *Lactobacillus casei* ATCC 393 as potential components of a liquid membrane or a liquid-liquid extraction process for lactic acid removal in an intensified fermentation process. Glucose consumption, lactic acid production, and cell growth were used as toxicity indicators. Physical properties of extractants and diluents were related to the molecular toxicity on the microorganism. These results show that mixtures of tri-iso-octylamine/dodecane and trioctylamine/dodecane at a proportion of 1:9 v/v have great potential to be used in liquid membranes or liquid-liquid extraction processes on hybrid fermentations with *Lactobacillus casei* ATCC 393.

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3.1.1 Introduction

Removal of organic acids through liquid membranes using tertiary amines has been the focus of several studies [1], especially for the recovery from fermentation systems. Succinic acid has been recovered using a hollow-fiber supported liquid membrane with a trialkylamine [2]. Citric acid has been removed using an emulsion liquid membrane with Alamine 336 [3]. Acetic acid has been separated by emulsion liquid membranes with Amberlite LA-2 (secondary amine), trioctylamine [4], and Aliquat 336 (quaternary ammonium salt) [5]. Lactic acid has been recovered by a supported liquid membrane with trioctylamine [6] and by emulsion liquid membranes with both tributyl phosphate and trioctylamine [7].

In the aforementioned perstraction and reactive liquid-liquid extraction processes, an extractant or carrier is mixed with a diluent [1,4,8,9]. Several characteristics are required from these extractants and solvents, for instance: low viscosity, a high difference of densities with the aqueous phase (that contains the solute), low melting point, medium interfacial tension, high hydrophobicity, thermal stability, low price and availability [10–12], within others. Additionally, toxicity levels of extractant and diluent on microorganisms are key factors to take into account with liquid membranes or liquid-liquid extractions applied for the removal of metabolites from fermentation systems.

Tertiary amines have commonly been used for organic acid removal owing to their high extraction availability, low water solubility and high selectivity [11,13–16] which is usually due to the formation of carboxylic acid-amine complexes [10,17]. Diluents allow for the adjustment of viscosity and density of the solvent phase [15], and on the other hand, provide high distribution coefficients [10]. Diluents can be inert or have an interaction with the extractant that influences its performance. An inert diluent can improve the physical extraction without affecting the transport mechanism [9] while an active diluent can have functional groups that interact with the carboxylic acid-amine complex solvating it in order to stabilize the complex [9,14].

Toxic effects of a solvent on microorganisms are related with accumulation in the cytoplasmic cell membrane. At this level, modifications are made in the functionality of the membrane proteins. Solvents go into and disrupt the lipid bilayer. These compounds generate rupture and metabolite leakage damage. Its effect may result in cell lysis and death [18–20]. The aforementioned toxic effects have been related to a physical property of the substance, the logarithm of the partition coefficient of the solvent in an equimolar mixture of n-octanol and water (L_{og} P_{ow}) [18,21–23]. As lower the Log P_{ow} is, the greater polarity and the toxicity of the solvent will be [22]. The solvent interacts with the microorganism mainly by two routes: direct contact between the cells and the

solvent into the aqueous-organic interface (phase toxicity) and by the organic solvent that is soluble in the aqueous broth (molecular toxicity) [19,20,24,25].

When a perstraction process is integrated with a fermentation process (hybrid process), the toxicity of the membrane phase (diluent/extractant) on the microorganisms can rise [10,16]. Thus, additional parameters have to be considered for designing the extraction or perstraction process including loading ratio, complexation equilibrium constant, stoichiometry and rate constant of the organic acid-amine complex [10,26]. In a hybrid process with in-situ product removal (ISPR) [27], either by reactive liquid-liquid extraction or supported liquid membranes, both molecular and phase toxicity affect cell growth and productivity. In order to reduce toxicity, filtration is used for removing biomass from the fermentation broth [25]. However, despite performing the product removal externally to the bioreactor, molecular toxicity can affect the microorganism.

Toxicity of several solvents has been tested on different lactic acid microorganisms. For instance, toxicity of n-dodecane, paraffin oil, chloroform, 1,1,1-trichloroethane, carbon tetra-chloride, ethyl laurate, n-dodecanol, tri-n-dodecylamine, and perfluorodecalin were tested on *Lactobacillus delbrueckii* ATCC 9649 [28]. Also, the toxicity of Hostarex A327, trihexylphosphate, pentaphosphine-dipentylester, diisotridecylamine, n-octanol, isodecanol, isotridecanol, oleyl alcohol and n-alkanes (C10–C13) were measured on *Rhizopus arrhizus* CCM 8109 [29]. Toxic effects of Imidazolium-based ionic liquids were tested on *Lactobacillus delbruekii* NRIC 1683 [24]. Toxicity of Tributylphosphate, tridodecylamine, dioctylamine, tri-n-octylamine, Alamine 336, Aliquat 336, 1-octanol, 1-decanol, 1-dodecanol, oleyl alcohol, n-octane, n-decane, dodecane and kerosene were evaluated on *Lactobacillus casei* NBMCC-1013 [20]. Almost all these studies (except [28,29]) are based on biomass growth and/or lactic acid production.

Also, it has been shown in the scientific literature that toxicity studies of the several organic solvents on different microorganisms have been contradictory [20], showing that the toxicity is highly related with the type and even the kind of strain of the microorganism. On the other hand, most of the studies have been focused on analyzing the lactic acid production and/or cell growth but rarely glucose consumption has been taken into account.

In this work, the molecular toxicity of three diluents (dodecane, dodecanol, and oleyl alcohol) and three carriers (trioctylamine, tri-iso-octylamine and Aliquat 336), with high potential to be used in lactic acid removal by perstraction or reactive liquid extraction was measured on the bacteria *Lactobacillus casei* ATCC 393. Also, the molecular toxicity of mixtures trioctylamine/n-dodecane

and tri-iso-octylamine/n-dodecane were evaluated at three different volumetric ratios. The toxic effects were analyzed by measuring biomass growth, lactic acid production, and glucose consumption.

3.1.2 Materials and methods

Molecular toxicity of n-dodecane (Merck, assay 99%), 1-dodecanol (Merck, assay 98%) and oleyl alcohol (Merck, assay 85%) as diluents and trioctylamine (TOA, Merck, assay 93%), tri-iso-octylamine (TiOA, Merck, assay 95%) and Aliquat 336 (Sigma-Aldrich) as extractants were evaluated separately on cell growth of the bacteria *Lactobacillus casei* ATCC 393 (Microbiologics) using MRS (Sharlau) culture media.

The experimental design for toxicity was first with pure carriers and solvents. For the solvent with the lowest toxicity, the experiments were carried out with amine (TOA, TiOA) concentrations at three levels in the range of concentration reported in the literature for liquid membrane or liquid extraction applications [6,26]. Aliquat 336 was not tested in mixtures with solvent due to its high toxicity. All experiments were carried out with duplicates.

In every single test for each pure substance, 100 mL of culture media was prepared and added into a 250 mL glass flask. Afterward, the organic compounds were added in order to achieve a volume ratio of 1:10 (organic compound:culture media). The six flasks, one for every organic compound (n-dodecane, dodecanol, oleyl alcohol, TOA, TiOA, Aliquat), were shacked using a magnetic stirrer (Velp Scientifica) at 120 rpm and constant temperature of 310 K in an incubator (Binder RI 115, ± 0.3 K) for 72 h (contact stage). Then, the agitation was stopped, each flask was transferred to separation funnels and kept in an oven at 310 K during 72 h for phase splitting (aqueous from the organic phase). Subsequently, the phases were separated.

Volumes of 7.5, 15, 22.5 and 30 mL of each aqueous phase, previously saturated with an organic phase (described above), were taken and mixed with fresh culture media to reach a total volume of 30 ml using Falcon centrifuge tubes of 50 mL. These volumes correspond to proportions of 25%, 50%, 75% and 100 vol%. Then, each culture media was inoculated at 5 vol% with *Lactobacillus casei* ATCC 393 from an inoculum of 12 h. One control (fermentation without toxic agents) was prepared using fresh culture media. At the beginning of the fermentation (previous to inoculum step, 0 h) and at the end (24 h) one sample was extracted from each Falcon tube (6 (mixtures) x 4 (levels) x 2 (duplicates) = 48 tubes) in order to quantify the glucose consumption, lactic acid and biomass production (in percentage referred to control fermentation). Bacterial growth was measured by

optical density (OD at the maximum wavelength, 540 nm) using a spectrophotometer (V1200, Mapada Instruments) and by dry cell weight method [30]. Lactic acid production and glucose consumption were measured by HPLC (ELITE LaChrom) using ORH-801 column (Chrom Tech) with a RI detector at 318.15 K using as a mobile phase a solution of 0.01 N H_2SO_4 (Merck, assay 95-97%) prepared with type 1 water (Barnstead™ Nanopure™). Every single Falcon tube was kept in an oven at 310 K for 24 h.

Trioctylamine and tri-iso-octylamine were mixed at concentrations of 10, 20 and 30 vol% with n-dodecane (0% of amine corresponds to previous experiments with pure dodecane) and its molecular toxicity level were tested following the aforementioned procedure using 30 mL of the aqueous phase (from the phase splitting) for the fermentation step. These concentration of the amine are typical for liquid membranes and liquid extraction [6,13,26]. As it is shown below, Oleyl alcohol and dodecanol were excluded due to their high toxicity compared to dodecane.

3.1.3 Results and discussion

Based on the classification of J. Marták *et al*. [29], organic solvents can be divided into three groups according to their toxicity level on microorganisms. Non-toxic, when the production rate is beyond 75%, medium toxicity when the production rate is between the 25% and 75%, and toxic when the production rate is less than 25% as compared with a control fermentation.

Molecular toxicity for pure solvents

Figures 1-3 show the relative lactic acid (LA) production, cell growth and glucose consumption of a fermentation broth composed of a mixture of fresh culture media and saturated culture media with the specific solvent at proportions of 25, 50, 75 and 100 vol%, (referred to the saturated culture media). Figures 1-3 are based on a culture media that were not in contact with the studied organic compounds (control fermentation) and for which the LA production, cell growth, and glucose consumptions were also measured. Taking into account the Marták criteria [29], dodecane was non-toxic for the four tested proportions. Cell growth and LA production were still 16% lower than the control fermentation at the four proportions, while glucose consumption was still 6% higher. For dodecane molecular toxicity test, the glucose concentration in the culture media increases after the contact stage due to the transport of water and some nutrients from the culture media to the organic phase. Thus, the glucose concentration of the saturated culture media was between 1.4% and 5.3% (SD = 1.5 – 7.6%), higher than the control fermentation, and in consequence, the glucose

consumption was promoted achieving values of glucose consumption slightly above the 100%. Taking into account a higher glucose consumption is expected an increase in biomass and LA production. However, they were till 10% lower but within the standard deviation (maximum *SD* = 11.2%). The toxicity of dodecane on the bacteria was low enough that it does not have an important effect on the measured fermentation variables.

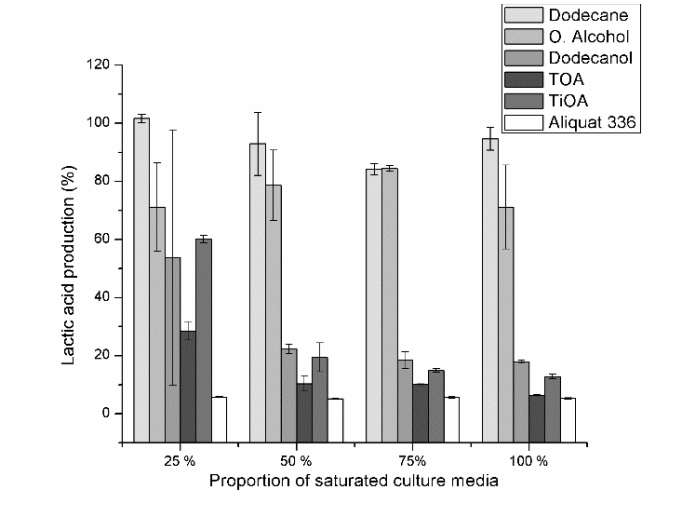

Figure 1. Relative lactic acid production for each pure substance at four proportions of the saturated culture media.

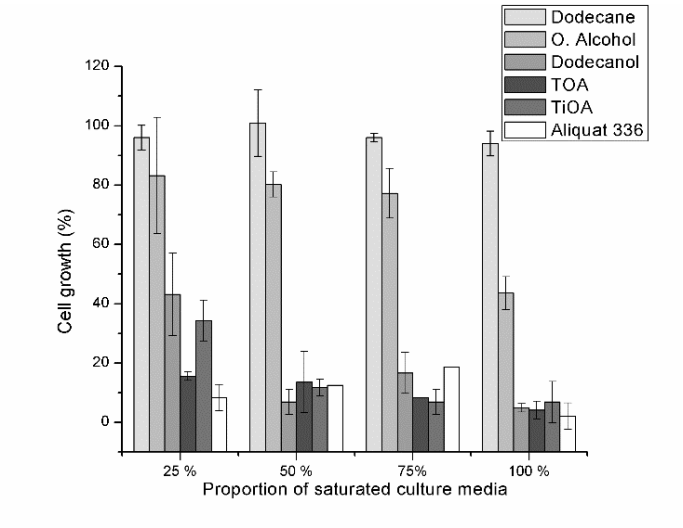

Figure 2. Relative cell growth by dry weight for each pure substance at four proportions of the saturated culture media.

Oleyl alcohol was non-toxic for volumetric proportions from 25 to 75% considering LA production, cell growth, and glucose consumption (with a minimum and maximum standard deviation of ±0.9 and ±19.6). However, it can be observed that for cell growth (Figure 2) at proportions from 25 to 75%, alcohol can be classified as medium toxic if the standard deviations are taking into account. Oleyl alcohol was medium toxic for a proportion of 100% based on LA production, cell growth, and glucose consumption (with a minimum and maximum standard deviation of ±5.6 and ±18). The glucose consumption (Figure 3) was reduced by 20% for 25, 50 and 75% proportions and it was reduced by 30% for a proportion of 100%. On the other hand, both cell growth and LA production decrease around 20% for proportions of 25, 50 and 75% and around 30% for a proportion of 100%. These results seem to indicate that the bacteria use glucose equitably to produce LA and biomass when oleyl alcohol is present.

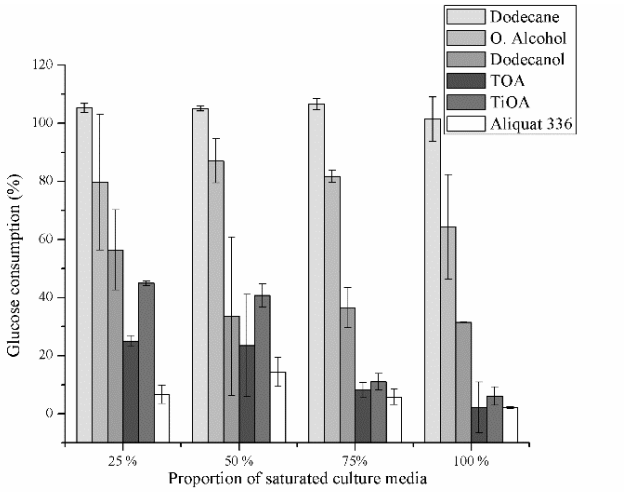

Figure 3. Relative glucose consumption for each pure substance at four proportions of the saturated culture media.

Dodecanol has a medium toxicity for a proportion of 25%, and it was toxic for the other proportions based on LA production and cell growth (Figure 1). However, glucose consumption was reduced around 40% for a proportion of 25% and around 60% for proportions of 50, 75% and 100%. Also, cell growth rate was reduced by 60% for a proportion of 25% and around 90% for proportions of 50, 75% and 100%. LA production was reduced around 50% for a proportion of 25% and around 80% for the other proportions. The aforementioned results show that the bacteria consumes more glucose to survive than the biomass and LA produced. It uses this glucose for maintenance and this behavior

is more intense at proportions above 50% where there are a lower cell growth and LA production as compared to the glucose consumption.

Aliquat 336 (a quaternary ammonium salt mixed with tertiary amines C8-C10-alkyl, octanol, and decanol) was toxic for all tested proportions, taking into account both LA produced and cell growth. LA produced was reduced by 95% as compared with control fermentation. The consumed glucose was reduced around 95% for volumetric proportions of 25, 75 and 100%, but it was reduced by 86% for a proportion of 50%. The cell growth was reduced around 95% for proportions of 25 and 100% and it was reduced by 88% for a proportion of 50%. The aforementioned results show that the bacteria use the consumed glucose to produce biomass but reduce its LA production to survive and for maintenance. For a proportion of 75% the cell growth was reduced by 82%. At this point, the bacteria produced an amount of LA in accordance with the consumed glucose but the relative fraction of biomass produced was higher than the consumed glucose. Aliquat 336 was the most toxic amine among tested extractants. Aliquat 336 contains trioctylmethylammonium chloride (TOMAC) that has a chloride ion in its structure and can interact with some substances from the MRS, such as sodium acetate and triammonium citrate to produce sodium chloride and ammonium chloride. Ammonium chloride is soluble in water and increases the medium acidity, whereas low pH can inhibit the lactic acid bacteria [11,16,31].

Trioctylamine (TOA) is toxic for the four volumetric proportions tested. Based on the cell growth, it is medium toxicity for a proportion of 25% in terms of LA production and it is toxic for the other proportions. However, this value of medium toxicity could be read as toxic considering the standard deviation. The consumed glucose is used to equitably produce LA and biomass for proportions of 75 and 100%, and as well for a proportion of 50% if the standard deviations of glucose consumption and cell growth are taking into account. For a proportion of 25%, the glucose consumption and LA production were reduced by 75% but cell growth was reduced by 85%, showing that the bacteria reduces its biomass production for maintenance.

Tri-iso-octylamine (TiOA) was the only extractant that exhibited a medium toxicity. It was medium toxic for a proportion of 25%, the other proportions and the other amines were toxic based on both, cell growth and LA production. TOA and TiOA are tertiary amines with the same structural formula thus it can be expected the same toxicity level. However, TiOA was less toxic than TOA. The *iso* structure into the alkyl group for TiOA can produce steric effects that probably reduces its toxic effects.

Although it is known that toxicity of organic solvents depend on the type of microorganism or even of its strain [10,29,34], generally the toxicity is related to the solvent polarity (related to Log P_{ow} values - Table 1) [1,19,24], and its water solubility [10,25]. Therefore, it is expected that dodecanol and oleyl alcohol, which are polar, were more toxic than dodecane (non-polar). The toxicity with the alkane is similar to other studies where alkanes were non-toxic or low toxic on bacteria [29,35]. For alcohols, it is known that its toxicity decreases as its long-chain increases [1,29,35,36]. Also, this study shows that oleyl alcohol (C18) is less toxic than dodecanol (C12) on the bacteria *Lactobacillus casei* ATCC 393 (Figure 2).

Table 1. Main physical properties of the tested solvents related with its toxicity on microorganisms.

Substance	Water solubility [g L^{-1}] at 298.15 K	Log P_{ow}	Surface tension [dyn cm^{-1}] at 298.15 K
Dodecanol	0.004	4.56	31.01
Oleyl alcohol	Insoluble	7.05	34.39
Dodecane	Practically insoluble	5.44	22.53
Aliquat 336	Practically insoluble	9.79[b]	39.53[b]
TOA	< 0.0001	9.46	32.50
TiOA	< 1[a]	9.25	29.19

Physical properties were calculated with ProPed-ICAS 14 software (CAPEC-DTU) using Marrero and Gani, Constantinou and Gani methods [32,33]. Water solubility was taken from MSDS. [a]Physical property at 293.15 K. [b]Physical property calculated for TOMAC using connectivity index neglecting ionic bond.

The logarithm of P_{ow} (Table 1) is a common parameter to study toxic effects on microorganisms [24,37]. A Log P_{ow} higher than 4, apparently does not have any effect on the biocatalytic activity of the cell [37]. However, a type of *Lactobacillus Delbruekii* is sensitive to organic solvents with Log P_{ow} between 1 to 4 [24]. Taking into account Log P_{ow} as a parameter of toxicity [24,37], the tested solvents can be considered to be low toxic, except dodecanol that it can be considered as non-toxic due to its Log P_{ow} value, nevertheless, another scientific literature have shown that Log P_{ow} is an unreliable parameter [1] to infer the toxicity level. However, other parameters as solubility and surface tension can affect the microbial growth (biomass and LA production, and glucose consumption).

Surface tension and water solubility have also been used to predict the toxic effect of the solvent on the microorganism. A high surface tension involves a rapid loss of cellular activity [19,37] where the cell membrane fluidity increases [19]. Also, the lower the solvent solubility in water is, the lower the probability of contact between the solvent and the microorganism will be. Thus, microorganisms will be less affected for the solvent presence into the culture media. For these reasons oleyl alcohol

is less toxic than dodecanol (water solubility in Table 1) in spite of having a similar surface tension. And dodecane has a low toxicity because it has a lower surface tension and lower water solubility than dodecanol and Oleyl alcohol.

In extractants, the higher the surface tension is the higher the toxicity on the bacteria will be, resulting Aliquat 336 with the highest toxicity in spite of being the extractant with the lowest water solubility. The lower toxicity of TiOA as compared with TOA is due to TOA has a higher surface tension and in consequence, it disturbs the cell membrane function as a barrier, as a matrix for enzymes and as energy transducer [23].

Molecular toxicity of selected mixtures

Figures 4-6 shows lactic acid production, cell growth and glucose consumption (respectively) for the culture media after contact with mixes of TiOA or TOA (less toxic amines) in dodecane (less toxic diluent) at volumetric proportions of 10, 20 and 30% of the respective tertiary amine in dodecane.

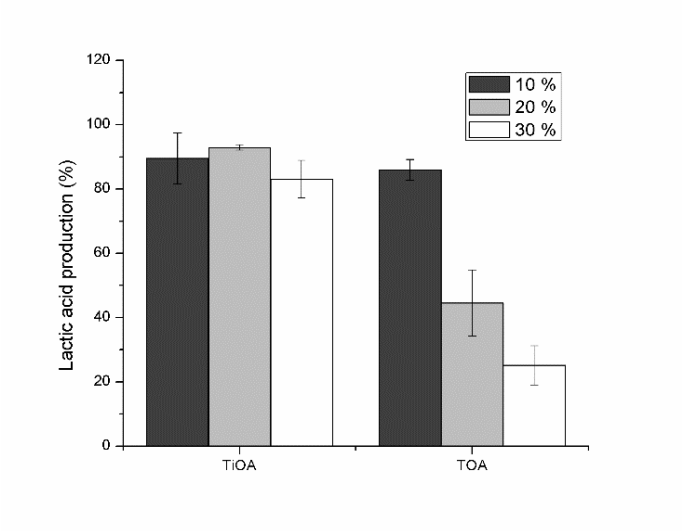

Figure 4. Lactic acid production for TiOA/dodecane and TOA/dodecane mixtures at three proportions of the tertiary amine.

TiOA mixed in n-dodecane was non-toxic at the three volumetric proportions for both, LA production and cell growth. For cell growth, the toxicity effect on the bacteria rises as the TiOA concentration increases, being non-toxic for volumetric proportions of 10 and 20% and medium toxic at 30%. There is a LA production according to the glucose consumption for three proportions of TiOA. However, as TiOA concentration rises, the cell growth decreases, perhaps in order to survive due to a high energy consumption of the bacteria trying to tolerate the organic solvent. These

microorganisms have developed several mechanisms to increase its tolerance for organics solvents, one of them involves the action of efflux pumps to expel the organic solvent cumulated into the membrane and another one involves to change the membrane rigidity in order to do it less permeable to the organic solvents [21]. Both mechanisms require an additional energy consumption.

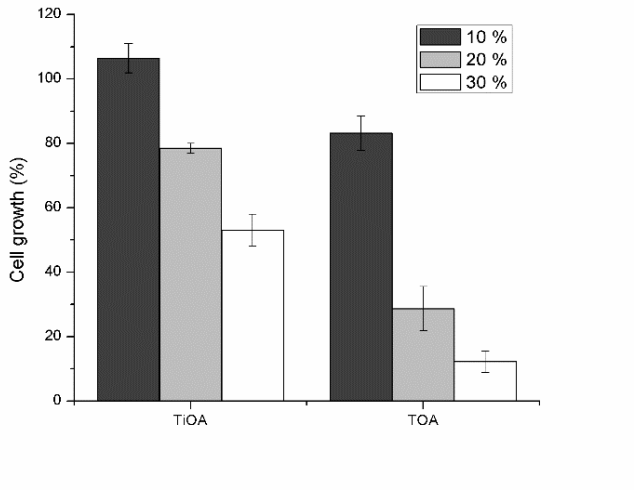

Figure 5. Cell growth by dry weight for TiOA/dodecane and TOA/dodecane mixtures at three proportions of the tertiary amine.

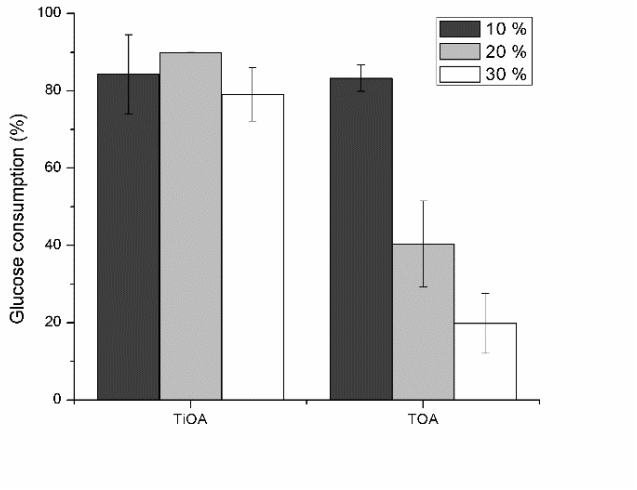

Figure 6. Glucose consumption for TiOA/dodecane and TOA/dodecane mixtures at three proportions of the tertiary amine.

TOA was non-toxic at a volumetric proportion of 10% for LA production and it was medium toxic for both cell growth and glucose consumption. It was medium toxicity at 20% of TOA and toxic at 30%. It means that the higher the TOA concentration is the higher the toxicity effect on the bacteria will be. For both proportions 20 and 30% of TOA, the consumed glucose was used, in higher proportion, to produce LA than biomass. LA production is proportional to the glucose consumed but the biomass production is reduced. This means that for every single cell the LA production is increased as compared to the control fermentation. The metabolic changes inside the cell do not clearly explain this behavior.

3.1.4 Conclusions

Taking into account cell growth, lactic acid production and glucose consumption, molecular toxicity level follows this order: dodecanol > oleyl alcohol > dodecane and for the extractants, the order is: Aliquat 336 > TOA > TiOA. Comparing the extractans with the diluents, extractants have higher molecular toxicity level. However, these extractants require to be mixed with a non-toxic diluent that ideally has a small effect on its extraction capacity.

Log P_{ow} is not a convenient property to evaluate the toxicity of the tested solvents. There is not an apparent relation between this parameter and the toxicity of the solvent on the bacteria.

Surface tension and water solubility of the solvents have a considerable influence on the toxicity on the microorganisms. Solvents with a high surface tension or water solubility are more toxic for the bacteria. It means, when a solvent is expected to be toxic for the microorganism, it is convenient to use an organic solvent with a low water solubility.

Tertiary amines, (TiOA and TOA) mixed in dodecane, although were medium toxic and toxic respectively, inhibited cell growth and promoted the LA production (at proportions of 20 and 30%). Conversely, TiOA at low concentration (10%) was non-toxic and promoted cell growth instead of LA production.

Both TiOA/dodecane and TOA/dodecane at a proportion of 10% have potential to be used in perstraction processes due to its low molecular toxicity level, where the lactic acid can be removed externally to the bioreactor from a fermentation broth free of biomass.

3.1.5 References

[1] Š. Schlosser, R. Kertész, J. Marták, Recovery and separation of organic acids by membrane-

based solvent extraction and pertraction, Sep. Purif. Technol. 41 (2005) 237–266. doi:10.1016/j.seppur.2004.07.019.

[2] S.-J. Li, H.-L. Chen, L. Zhang, Recovery of fumaric acid by hollow-fiber supported liquid membrane with strip dispersion using trialkylamine carrier, Sep. Purif. Technol. 66 (2009) 25–34. doi:10.1016/j.seppur.2008.12.004.

[3] B. Yordanov, L. Boyadzhiev, Pertraction of citric acid by means of emulsion liquid membranes, J. Memb. Sci. 238 (2004) 191–197. doi:10.1016/j.memsci.2004.04.004.

[4] S.C. Lee, K.-S. Hyun, Development of an emulsion liquid membrane system for separation of acetic acid from succinic acid, J. Memb. Sci. 350 (2010) 333–339. doi:10.1016/j.memsci.2010.01.008.

[5] S.C. Lee, Development of an emulsion liquid membrane system for removal of acetic acid from xylose and sulfuric acid in a simulated hemicellulosic hydrolysate, Sep. Purif. Technol. 118 (2013) 540–546. doi:10.1016/j.seppur.2013.07.032.

[6] R.-S. Juang, S.-H. Lee, R.-C. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239. doi:10.1016/S0376-7388(97)00206-8.

[7] J. Yuanli, Modeling of the permeation swelling of emulsion during lactic acid extraction by liquid surfactant membranes, J. Memb. Sci. 191 (2001) 215–223. doi:10.1016/S0376-7388(01)00470-7.

[8] J. Berrios, D.L. Pyle, G. Aroca, Gibberellic acid extraction from aqueous solutions and fermentation broths by using emulsion liquid membranes, J. Memb. Sci. 348 (2010) 91–98. doi:10.1016/j.memsci.2009.10.040.

[9] G. Kyuchoukov, A. Labbaci, J. Albet, J. Molinier, Simultaneous Influence of Active and “Inert” Diluents on the Extraction of Lactic Acid by Means of Tri- n -octylamine (TOA) and Tri- iso -octylamine (TIOA), Ind. Eng. Chem. Res. 45 (2006) 503–510. doi:10.1021/ie050912f.

[10] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[11] C.S. López-Garzón, A.J.J. Straathof, Recovery of carboxylic acids produced by fermentation, Biotechnol. Adv. 32 (2014) 873–904. doi:10.1016/j.biotechadv.2014.04.002.

[12] M. Jung, B. Schierbaum, H. Vogel, Extraction of Carboxylic Acids from Aqueous Solutions with the Extractant System Alcohol/Trin-Alkylamines, Chem. Eng. Technol. 23 (2000) 70–74. doi:10.1002/(SICI)1521-4125(200001)23:1<70::AID-CEAT70>3.0.CO;2-O.

[13] B. Choudhury, T. Swaminathan, Lactic acid extraction with trioctyl amine, Bioprocess Eng. 19 (1998) 317. doi:10.1007/s004490050526.

[14] D.H. Han, W.H. Hong, Water-Enhanced Solubilities of Lactic Acid in Reactive Extraction Using Trioctylamine/Various Active Diluents Systems, Sep. Sci. Technol. 33 (1998) 271–281. doi:10.1080/01496399808544768.

[15] J.A. Tamada, A.S. Kertes, C.J. King, Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, Ind. Eng. Chem. Res. 29 (1990) 1319–1326. doi:10.1021/ie00103a035.

[16] D. Yankov, J. Molinier, G. Kyuchoukov, J. Albet, G. Malmary, Improvement of the Lactic Acid Extraction . Extraction From Aqueous Solutions and Simulated Fermentation Broth by Means of Mixed Extractant and TOA , Partially Loaded with HCl, Chem. Biochem. Eng. Q. 19 (2005) 17–24. http://silverstripe.fkit.hr/cabeq/assets/Uploads/Cabeq-2005-01-3.pdf.

[17] M. Hossain, Mass Transfer Investigation of Organic Acid Exatraction with Trioctylamine and Aliquat 336 Dissolved in Various Solvents, in: Mass Transf. Multiph. Syst. Its Appl., InTech, 2011: pp. 367–388. doi:10.5772/15276.

[18] J.L. Ramos, E. Duque, M.-T. Gallegos, P. Godoy, M.I. Ramos-González, A. Rojas, W. Terán, A. Segura, Mechanisms of Solvent Tolerance in Gram-Negative Bacteria, Annu. Rev. Microbiol. 56 (2002) 743–768. doi:10.1146/annurev.micro.56.012302.161038.

[19] Z. Gu, B.A. Glatz, C.E. Glatz, Propionic acid production by extractive fermentation. I. Solvent considerations, Biotechnol. Bioeng. 57 (1998) 454–461. doi:10.1002/(SICI)1097-0290(19980220)57:4<454::AID-BIT9>3.0.CO;2-L.

[20] N.A. Marinova, D.S. Yankov, Toxicity of some solvents and extractants towards Lactobacillus casei cells, Bulg. Chem. Commun. 41 (2009) 368–373. http://www.bcc.bas.bg/BCC_Volumes/Volume_41_Number_4_2009/Volume_41_Number_4_2009_PDF/2916-AC.pdf.

[21] P. Fernandes, Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics, Int. J. Antimicrob. Agents. 22 (2003) 211–216. doi:10.1016/S0924-8579(03)00209-7.

[22] Y. Sardessai, S. Bhosle, Tolerance of bacteria to organic solvents, Res. Microbiol. 153 (2002) 263–268. doi:10.1016/S0923-2508(02)01319-0.

[23] S. Isken, J.A.M. de Bont, Bacteria tolerant to organic solvents, Extremophiles. 2 (1998) 229–238. doi:10.1007/s007920050065.

[24] M. Matsumoto, K. Mochiduki, K. Kondo, Toxicity of ionic liquids and organic solvents to lactic acid-producing bacteria, J. Biosci. Bioeng. 98 (2004) 344–347. doi:10.1016/S1389-1723(04)00293-2.

[25] V.M. Yabannavar, D.I.C. Wang, Strategies for reducing solvent toxicity in extractive fermentations, Biotechnol. Bioeng. 37 (1991) 716–722. doi:10.1002/bit.260370805.

[26] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria for Trioctylamine/1-Dodecanol/Lactic Acid/Water System at 306.1, 310.1 and 316.1 K: Experimental Data and Prediction, J. Chem. Eng. Data. 61 (2016) 2269–2276. doi:10.1021/acs.jced.5b00955.

[27] J.M. Woodley, M. Bisschops, A.J.J. Straathof, M. Ottens, Future directions for in-situ product removal (ISPR), J. Chem. Technol. Biotechnol. 83 (2008) 121–123. doi:10.1002/jctb.1790.

[28] R. Bar, J.L. Gainer, Acid Fermentation in Water-Organic Solvent Two-Liquid Phase Systems, Biotechnol. Prog. 3 (1987) 109–114. doi:10.1002/btpr.5420030208.

[29] J. Marták, E. Sabolová, Š. Schlosser, M. Rosenberg, L. Kristofíková, Toxicity of organic solvents used in situ in fermentation of lactic acid by Rhizopus arrhizus, Biotechnol. Tech. 11 (1997) 71–75. doi:10.1023/A:1018408220465.

[30] K. Hayakawa, H. Sansawa, T. Nagamune, I. Endo, High density culture of lactobacillus casei by a Cross-Flow culture method based on kinetic properties of the microorganism, J. Ferment. Bioeng. 70 (1990) 404–408. doi:10.1016/0922-338X(90)90122-D.

[31] K. Hetényi, Á. Németh, B. Sevella, Role of pH-regulation in lactic acid fermentation: Second steps in a process improvement, Chem. Eng. Process. Process Intensif. 50 (2011) 293–299. doi:10.1016/j.cep.2011.01.008.

[32] J. Marrero, R. Gani, Group-contribution based estimation of pure component properties, Fluid Phase Equilib. 183–184 (2001) 183–208. doi:10.1016/S0378-3812(01)00431-9.

[33] L. Constantinou, R. Gani, New group contribution method for estimating properties of pure compounds, AIChE J. 40 (1994) 1697–1710. doi:10.1002/aic.690401011.

[34] M.J. Playne, B.R. Smith, Toxicity of organic extraction reagents to anaerobic bacteria, Biotechnol. Bioeng. 25 (1983) 1251–1265. doi:10.1002/bit.260250508.

[35] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71. doi:10.1016/j.bej.2004.03.006.

[36] R. Chen, Y.Y. Lee, Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass, Appl. Biochem. Biotechnol. 63–65 (1997) 435–448. doi:10.1007/BF02920444.

[37] V.M. Yabannavar, D.I.C. Wang, Bioreactor System with Solvent Extraction for Organic Acid Production, Ann. N. Y. Acad. Sci. 506 (1987) 523–535. doi:10.1111/j.1749-6632.1987.tb23847.x.

3.2 Liquid-liquid equilibrium and molecular toxicity of active and inert diluents of the organic mixture tri-iso-octylamine/dodecanol/dodecane as a potential liquid membrane for lactic acid removal[4]

Abstract

Lactic acid can be in-situ removed from a fermentation broth through reactive liquid extraction or a liquid membrane to enhance the fermentation process. The organic mixture tri-iso-octylamine (TiOA)/dodecanol/dodecane at 10 vol% of the amine is a potential organic mixture for lactic acid removal. Liquid-liquid equilibria with lactic acid aqueous solutions and molecular toxicity on the bacteria *Lactobacillus casei* ATCC 393 were measured with several dodecanol proportions in dodecane (0 to 90 vol%) and 10 vol% TiOA as potential solvents or membrane phases for LA removal from a fermentation broth. Effects of the organic phase on the bacteria as cell growth, biomass production, glucose consumption, productivity and product to biomass yield are analyzed. Dodecanol increases the lactic acid chemical equilibrium constant for the liquid-liquid equilibria, while increases the molecular toxicity on the bacteria. However, for dodecanol concentrations from 30 to 40 vol% the value of the chemical equilibrium constant is high enough for lactic acid distribution between the phases and its toxicity is low enough on the bacteria, making a proper range of dodecanol concentrations for lactic acid removal. Also, the distribution coefficient and the chemical equilibrium constant are fitted as function of the dodecanol concentration in the organic mixture.

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3.2.1 Introduction

Lactic acid (LA) is a commodity chemical [1,2] which can be obtained by chemical synthesis or by carbohydrate fermentation [3–5]. The 90% of the LA is produced by biotechnological route [4,6]. However, in the conventional process for LA production, the bacterial growth is inhibited on the one hand by LA, achieving low concentrations of LA, and on the other hand by substrate, limiting initial substrate concentration within the bioreactor [1,7,8]. Besides, this process requires multiple separation steps for recovery and purification of the LA from the fermentation broth [1,2,9]. The whole LA production process is expensive [1,2], and 50% of the total cost is due to the separation and final purification steps [1,2,9].

LA biotechnological production requires an enhancement on its production process that avoid the common drawbacks of the conventional process, which are cell growth inhibition (by product and substrate), the use of neutralizers, low yield and productivity, and high overall costs of the process [2]. Several separation technologies, such as solvent extraction, adsorption (with and without ion exchange), distillation, filtration, ultrafiltration, nanofiltration, electrodialysis, reverse osmosis, evaporation, crystallization, and liquid membranes have been tested for LA recovery from fermentation broths as promising alternatives over the conventional process [1,9,10].

In-situ LA removal using one or several of the aforementioned separation technologies is a potential strategy to overcome the typical drawbacks of LA production [1,10]. In-situ LA removal reduces end-product inhibition [1] and contributes to the fed-batch or continuous operation of the fermentation [7]. One of the most extensively studied separation technologies for LA removal has been reactive extraction [11–16]. However, this process requires high amounts of solvent and the use of back-extraction for regeneration of the solvent [8,17].

Perstraction is a liquid membrane process which can use the same solvent (diluent and extractant) of reactive extraction as membrane phase [18]. It does not require high amounts of solvent, and the extraction and back-extraction processes are combined in a single device [17,18]. This process is also called liquid membrane, and several studies using this membrane technology have been focused on LA removal [19–27].

Usually, the selection of the solvent, for either reactive extraction or liquid membranes, is based on the ability of the solvent to enhance the LA removal from the fermentation broth and the toxic effect of the solvent on the specific microorganism in the fermentation broth [28,29]. However, most of the solvents used for LA removal from fermentation broths are toxic on the specific microorganism [28].

There are two kinds of toxicity of the solvent on the microorganism depending on the type of contact between the solvent and the microorganism. On the one hand, molecular toxicity is due to the soluble portion of the solvent in the fermentation broth. On the other hand, phase level toxicity is due to the presence of two phases in the fermentation process, solvent phase and fermentation broth phase [30,31]. For in-situ LA removal using reactive extraction or liquid membranes, both levels of toxicity can affect the microorganism. However, filtration is usually used for removing biomass from the fermentation broth to avoid phase toxicity on the microorganism [32]. However, performing biomass filtration and product removal externally to the bioreactor will leave molecular toxicity to occur on the microorganism [33]. There are several cellular responses due to toxic organic solvents, such as stress [34], changes in cell morphology (filamentation) [34,35], cell surface modification (hydrophobic shift) [34], cell membrane adaptions (membrane fluidity) [34,35] and solvent excretion through efflux pumps [34,35], among others. Most of these cellular responses are energy demanding [36].

Single alcohols and tertiary amines mixed within alkanes or alcohols have extensively studied for LA removal because they have shown high extraction capabilities [11–16,22,37–39]. The liquid-liquid equilibrium of the mixture tri-octylamine at 0.8 M (around 23 mol%) in 1-dodecanol with LA aqueous solutions have shown a high value of the chemical equilibrium constant [12], becoming it as a potential solvent for reactive extraction or potential membrane phase for liquid membrane processes. However, toxicity studies are required for the selection of these organic compounds in an in-situ LA removal process either by liquid-liquid extraction or liquid membrane processes.

Molecular toxicity of pure 1-dodecanol, n-dodecane, oleyl alcohol, Aliquiat 336, tri-octylamine and tri-iso-octylamine (TiOA) were tested in a previous work [33], showing that n-dodecane is non-toxic on the *Lactobacillus casei* ATCC 393 (probiotic and homofermentative bacteria which the main metabolite is lactic acid). Depending on the concentration of oleyl alcohol within the fermentation broth, it can be classified between non-toxic or with medium toxicity. 1-dodecanol has medium toxicity, and the tertiary amines are toxic on the bacteria. However, tri-iso-octylamine at a low concentration within the fermentation broth (concentrations of TiOA 50% lower than saturation point on the fermentation broth) has medium toxicity. Also, the toxicity of the mixture TiOA/dodecane on the same bacteria was tested at 10, 20 and 30 vol% of TiOA (5.40, 11.38 and 18.04 mol%) showing that the mixture at 5.40 and 11.38 mol% is non-toxic and at 18.04 mol% is medium toxic [33].

Additionally, the liquid-liquid equilibria (LLE) of a mixture of TiOA within n-dodecane and another mixture of TiOA within 1-dodecanol, both at 10 vol% of TiOA (5.40 and 5.34 mol%, respectively) were previously carried out[40]. The mixture TiOA/dodecane showed a low capability to LA removal because this mixture does not promote the chemical reaction between the amine and the LA, while the mixture TiOA/dodecanol enhances LA removal due to dodecanol stabilizes the LA formed complex by solvation.

Summarizing, if pure Dodecane is used the molecular toxicity is low but also the LA removal is poor. If dodecanol is used there is a high LA removal but also molecular toxicity is high. Thus, a mixture of dodecane and dodecanol with TiOA could be a good compromise between LLE and molecular toxicity. However, the toxicity of the mixture TiOA/dodecanol/dodecane, at several proportions of dodecanol, on the *Lactobacillus casei* ATCC 393 bacteria and the LLE have been not tested.

In this work, it was measured the LLE (organic/aqueous LA) and molecular toxicity on the *Lactobacillus casei* ATCC 393 at several ratios of dodecanol:dodecane with 10 vol% of TiOA (5.4 to 5.34 mol%). Dodecanol concentrations between 0 to 90 vol% (0 to 94.66 mol%) were used. Correlations for prediction of the distribution coefficient and the chemical equilibrium constant are proposed as a function of the dodecanol concentration within the TiOA/dodecanol/dodecane mixture.

3.2.2 Experimental section

Materials

Table 1. Chemicals used for the experiments. Physicochemical properties were taken from their respective MSDS of the supplier (each one at room temperature).

Name	CAS	Supplier	Molecular weight	Density ($g \cdot mL^{-1}$)	Purity (wt%)
Tri-iso-octylamine	25549-16-0	Merck	353.68	0.8	95
n-dodecane	112-40-3	Merck	170.34	0.75	99
1-dodecanol	112-53-8	Merck	186.33	0.83	98
L(+)-lactic acid	79-33-4	Scharlau	90.08	1.206	88-92
Sodium hydroxide	1310-73-2	Merck	40.00	2.13	99

Tri-iso-octylamine, n-dodecane (assay 99%) and 1-dodecanol (assay 98%) have lower water solubility than 1 $g \cdot L^{-1}$ at 20 °C, insoluble at 25 °C, and 0.004 $g \cdot L^{-1}$ at 20 °C, respectively. L(+)-lactic acid was supplied by Panreac Química S.A.U. (assay 88.0-92.0%). The purity of L(+)-lactic acid

(extra pure) was assessed by titration with NaOH (assay ≥ 99.0%) using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). A stock solution of lactic acid (150 g/L) was heated at 90 °C under total reflux between 8-10 hours for dimer hydrolysis [15,41], and afterward, the lactic acid concentration was measured by titration. Type I water was used for all aqueous solutions (Barnstead™ Nanopure™). All chemicals used are listed in Table 1. *Lactobacillus casei* ATCC 393 (Microbiologics) was used for fermentations with MRS broth (Scharlau) as culture media.

Liquid-liquid equilibria experiments (LLE)

LLE experiments for organic phases with 10 vol% (5.40 to 5.34 mol%) of TiOA (extractant or carrier) in n-dodecane (inert diluent) and 1-dodecanol (active diluent) with concentrations of 1-dodecanol of 10, 20, 30 and 50 vol% were performed (10.62, 21,22, 31.79 and 52.85 mol%). For the LLE experiments and each of the above organic phases, 5 vials of 10 mL were filled with 2 mL of an aqueous solution of LA, at concentrations between 1 and 38 g/L (0.0166 and 0.6465 mol%). Then, 2 mL of the organic phase was added to each vial. The experimental procedure was performed at a constant temperature (37 °C) and consist in the next three steps [12]: agitation, decantation, and sampling. First, all vials were shaken at 180 rpm (Wiseshake Shot-1D, Wisd) with an incubator (Wisd, ± 0.6 K) during 72 h. Afterward, the agitation was stopped, and the vials were kept into the incubator (decantation step) during 72 h. One sample of the aqueous solution was taken from every single vial, and its lactic acid concentration was measured by high-performance liquid chromatography (HPLC ELITE LaChrom). For HPLC analysis, it was used an ORH-801 column (Chrom Tech) with a solution of 0.01 N H_2SO_4 (Merck, assay 95-97%) for the mobile phase, and a RI detector at 45 °C. The LA concentration in equilibrium within the organic phase was calculated by a material balance [12].

The measured LA concentrations at equilibrium were used to fit the distribution coefficient (eq 1) and the chemical equilibrium constant (eq 2) based on a LLE model developed in previous works [12,40]. Brackets in equations 1 and 2 represent molar concentrations

$$K_D = \frac{[LA]_{org}}{[LA]_{aq}} \tag{1}$$

$$K_E = \frac{[(LA)(TiOA)]_{org}}{[LA]_{org}[TiOA]_{org}} \tag{2}$$

The LA in the organic phase can be as free LA, which comes from the aqueous phase by diffusion, and as LA-TiOA complex, which is the LA that reacts with the amine both the organic-aqueous interface and the bulk of organic phase [12,40]. For the distribution coefficient, the LA concentration in the organic phase corresponds to the LA in its free form.

Molecular toxicity test

Toxicity of TiOA in 1-dodecanol at 5.34 mol% of TiOA was measured on the bacteria *Lactobacillus casei* ATCC 393 as shown elsewhere [33]. MRS broth was prepared according to the manufacturer instructions (Scharlau). 3 mL of the organic phase was added in a falcon tube with 30 mL of sterile MRS. The Falcon tube was shaken by 72 h at 180 rpm (Wiseshake Shot-1D, Wisd) at 37 °C into a bod incubator (Wisd, ± 0.6 K). Afterward, the shaker was stopped, the falcon tubes were kept in the incubator at the same temperature for 72 h to reach phase splitting (aqueous phase from the organic phase). Then, the aqueous phases (culture media saturated with the organic phase) were separated from the organic phase by splitting. Each culture media within a new sterile falcon tube was inoculated at 5 vol% from a culture of 12 h.

One control fermentation was prepared using fresh culture media (MRS without contact with the organic phase). The fermentation of each inoculated falcon tube was kept into a bod incubator (Wisd, ± 0.6 K) for 24 h at 37 °C. Samples from each falcon tube were taken at the beginning (0 h) and at the end (24 h) of each fermentation for measuring the percentage of bacterial growth, LA production and glucose consumption based on the control fermentation. Bacterial growth was measured by dry cell weight method [33]. LA production and glucose consumption were measured by HPLC using the same method of LA measurements in the LLE experiments.

The aforementioned procedure also was used to test the molecular toxicity of organic phases with 10 vol% of TiOA (5.40 to 5.34 mol%) in n-dodecane and at 1-dodecanol proportions of 10, 20, 30, 40, 50 and 90 vol% (0, 10.62, 21.22, 31.79, 42.33, 52.85 and 94.66 mol%, respectively). All experiments were performed by duplicate.

3.2.3 Results and discussion

Liquid-liquid equilibria

The distribution coefficient and the chemical equilibrium constant for the LLE of aqueous LA solutions in contact with tri-iso-octylamine/n-dodecane/1-dodecanol at 37 ºC increases as the concentration of dodecanol in the organic mixture rises (Figure 1). In the organic phase, there was

always TiOA at 10 vol% (5.40 to 5.34 mol%) and the proportion dodecane to dodecanol varied. Dodecane is an inert diluent and dodecanol is an active diluent [40]. Dodecanol provides a solvation shell for the free LA and for the LA complex in the organic phase [40], which stabilizes the acid-amine complex. Therefore, the availability of the dodecanol molecules to solvate is enhanced with an increase of dodecanol concentration in the organic phase.

The stoichiometric ratio (amine:acid) 1:1 is the most common for these systems where a tertiary amine is involved and especially when the LA concentration is low (lower than the stoichiometric concentration [40]). Therefore, it was used a stoichiometric ratio of 1:1 for fitting the values of K_D and K_E for all dodecanol concentrations from 0 to 94.66 mol% (Figure 1) using a previous LLE model [40]. For the organic mixture without dodecanol there is not complex formation, and the value of K_E is zero [40].

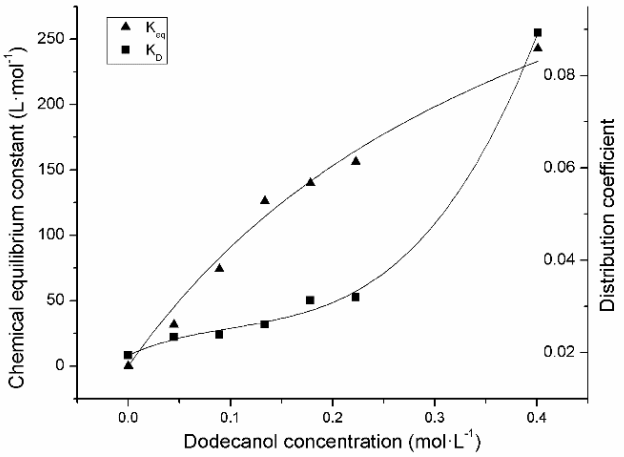


Figure 1. Fitted values of the distribution coefficient (squares) and the chemical equilibrium constants (triangles) with its respective correlations (continuous line) using eqs 3 and 4, respectively. Values of K_D and K_E at 0, 42.33 and 94.66 mol% of dodecanol were taking from a previous work [40].

The increase of the K_E with dodecanol concentration was monotonic, while for the K_D it does not show a proportional increase (Figure 1). For K_D it was used a third-grade polynomial fit (eq 3) while for the K_E it was used eq 4, which is a Langmuir equation. For both fits, a molar concentration of dodecanol was used. The corresponding fitted parameters are presented in Table 2 with their corresponding correlation coefficients.

$$K_D = a_3 \cdot [DOH]^3 + a_2 \cdot [DOH]^2 + a_1 \cdot [DOH] + a_0 \quad (3)$$

$$K_E = K_1 \frac{K_2[DOH]}{1 + K_2[DOH]} \quad (4)$$

Generally, in systems that used tertiary amines, most of the organic acid within the organic phase is due to a LA-Amine complex that it is formed with the tertiary amine [12,40]. Therefore, the amount of free LA within the organic phase in equilibrium is very small as compared to the LA-TiOA complex [40]. Thus, K_E provides more valuable information to determine which organic mixture provides a better capacity to remove the LA than K_D.

Table 2. Fitted parameters of eqs 3 and 4 with its corresponding correlation coefficients.

R^2	a_3 (L·mol^{-1})^3	a_2 (L·mol^{-1})^2	a_1 (L·mol^{-1})	a_0
0.9977	1.9987	-0.6186	0.1010	0.0194
R^2	K_1 (L·mol^{-1})	K_2 (L·mol^{-1})		
0.9863	485.18	2.3060		

Molecular toxicity test

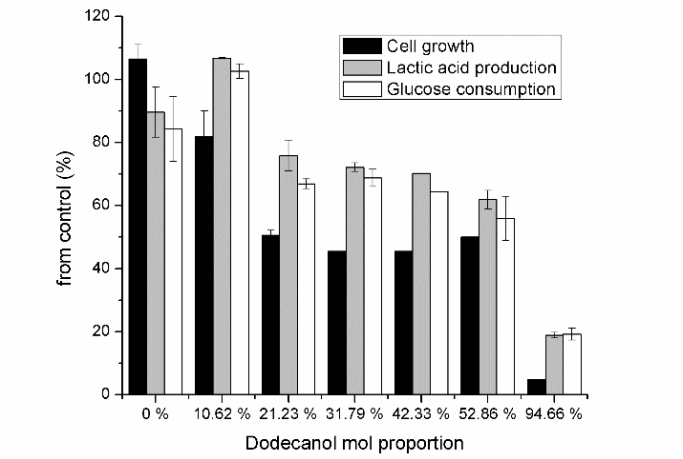


Figure. 2. Relative cell growth, lactic acid production and glucose consumption as compared to a control fermentation of the saturated media with TiOA/dodecanol/dodecane at 10 vol% of TiOA (5.4 to 5.34 mol%) and at proportions of dodecanol of 0, 10.62, 21.22, 31.79, 42.33, 52.85 and 94.66 mol% at 37 °C. Molecular toxicity for dodecanol proportion of 0 mol% was previously reported [33].

Molecular toxicity of the mixtures TiOA/dodecanol/dodecane at concentrations of dodecanol from 0 to 94.66 mol% on the bacteria *Lactobacillus casei* ATCC 393 is shown in Figure 2. The level of toxicity was classified according to J. Marták *et al.* [29], where the toxicity level is divided into three

categories. Non-toxic, when the production rate is beyond 75%, medium toxicity when the production rate is between the 25% and 75% and toxic when the production rate is less than 25% as compared with control fermentation.

Surface tension and water solubility are properties that can be related to toxicity levels of the solvent on a specific microorganism [33]. The toxicity of solvents increases as its surface tension rises because a high surface tension leads to a rapid loss of cellular activity [28,42]. On the other hand, the higher the water solubility of the solvent, the higher the probability of contact between the bacteria and the organic solvent [33]. The surface tension of dodecanol and dodecane are 31.01 and 22.53 dyn/cm (at 25 °C), respectively, and its water solubility for dodecanol is 0.004 g/L (at 25 °C), and dodecane is practically insoluble. Both physical properties are higher for dodecanol than for dodecane. Therefore, it is expected an increase of toxicity as the dodecanol concentration within the organic phase increases.

The mixture TiOA/dodecanol/dodecane at 0 mol% of dodecanol (Figure 2) is non-toxic taking into account cell growth, lactic acid production, and glucose consumption. Cell growth is not affected by the presence of the solubilized organic compounds of the organic phase into the culture media. However, LA production and glucose consumption slight decreases compared with control fermentation. Glucose consumption is 84.31%, while LA production is 89.54% of the values of the control fermentation. The bacteria response to the presence of the organic solvents, within the fermentation broth, in several ways [34,35] such as hydrophobic shift on cell surface to repel hydrophobic compounds [34], and homeoviscous adaptation that increases membrane rigidity because the degree of membrane fatty acid saturation also increases [34]. Perhaps these effects may affect the mechanism of glucose transport through the membrane cell or phosphotransferase system for sugars (PTS), making the glucose consumption of the bacteria lower than the control fermentation. The bacteria probably does not have enough ATP to support this mechanism.

The mixture TiOA/dodecanol/dodecane with 10.62 mol% of dodecanol is non-toxic, and mixtures with concentrations of dodecanol from 21.22 to 52.85 mol% are medium toxicity. For the organic mixtures TiOA/dodecanol/dodecane with dodecanol proportions from 10.62 to 52.86 mol%, the lactic acid production is around 5% higher than glucose consumption, while the biomass production is lower (between 6 and 25% below) than the LA production and the glucose consumption. Some of the responses of the cells to the toxic compounds are activation of efflux pumps, change the morphology, surface adaptations, among others, which are energy demanding [36]. Thus, it is probable that this additional energy requirement of the cells, as a mechanism to adapt or survive to

toxic compounds, directly affects the cell growth. The energy is used by the cell to activate one of several mechanisms to tolerate the toxic compounds within the fermentation broth instead to use it entirely for cell growth.

The mixture TiOA/dodecanol/dodecane at 94.66 mol% of dodecanol is toxic for cell growth, LA production, and glucose consumption according to the results showed in Figure 2. The glucose consumption is reduced as compared to control fermentation, probably because the glucose transport is affected by the hydrophobic shift on surface cell and homeoviscous adaptation in membrane cell, as responses of the bacteria to the toxic compounds. Glucose consumption is affected in the same proportion than LA production (around 19%). However, the cell growth is affected in a higher proportion achieving a bacterial growing of 4% of the one in the control fermentation. The low biomass production can be due to the bacteria kept the consumed glucose for maintenance instead of biomass production. Thus, the bacteria requires additional energy to use the efflux pumps to take out the toxic solvent from the bacteria increasing energy requirement on the bacteria. Therefore, the cells reduce the energy consumption for biomass production.

In Figure 2, it can be observed that for dodecanol concentration of 94.66 mol% (which means 0 mol% of dodecane) in the mixture TiOA/dodecanol/dodecane, the cell growth is lower than LA production, while for a dodecanol proportion of 0 mol% (which corresponds to 94.6 mol% of dodecane) the cell growth is higher than LA production. Therefore, the effect of that the LA production is promoted instead cell growth can be attributed to the presence of dodecanol within the fermentation broth, while the effect that cell growth is promoted instead LA production can be attributed to the presence of dodecane within the fermentation broth. Additionally, it is observed that for dodecanol concentrations from 10.62 to 52.85 mol%, the LA production is promoted instead the cell growth in spite of that dodecane is also present within the fermentation broth (Figure 2). It means that the dodecanol effect on the bacteria is stronger than the effect of dodecane on the bacteria.

Most of the published literature on molecular toxicity of organic compound on specific bacteria have shown in their results the toxic effect on cell growth. Only a few ones have taken into account both, the main metabolite production and the substrate consumption. In this work, it is observed that evaluating these three parameters is important to determine whether cell growth or main metabolite production is promoted. However, in spite of these results it is still unclear how these organic compounds interact within the bacteria or on its metabolism which could be explore in the future.

The relative LA productivity between the LA productivity of the fermentation saturated with the organic phase and the LA productivity of the control fermentation is around the unit for dodecanol

concentrations between 0 to 21.22 mol%. However, the fermentation saturated with the organic phase at 10.62 mol% of dodecanol leads to the maximum relative LA productivity, being around 1.4 (Figure 3). This effect is due to dodecanol at this concentration promotes LA production instead of cell growth. Also, the toxic effects on the bacteria are low, and cell growth, LA production, and glucose consumption are similar to the control fermentation. At this dodecanol concentration, the cell growth is reduced around 18% as compared with the control fermentation (Figure 2), while the LA production is increased around 7% (Figure 2). For dodecanol concentrations between 21.22 to 94.66 mol%, the relative LA productivity is lower than the unit and decreases as dodecanol concentration rises due to the toxic effect of the dodecanol on the bacteria (Figure 3).

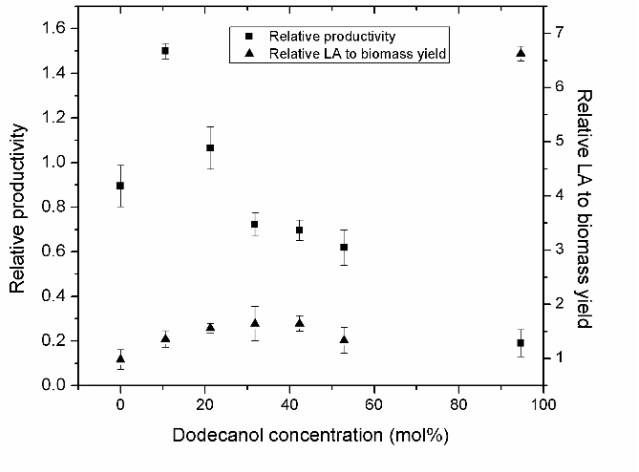


Figure 3. Effect of the dodecanol concentration on the organic phase (TiOA/dodecanol/dodecane) on the productivity ratio (squares) and on yield lactic acid to biomass ratio (triangles). Both are the ratio of the fermentations saturated of the organic phase with the control.

The relative LA to biomass yield is higher than the unit at all dodecanol concentrations (Figure 3), except for a dodecanol concentration of 0 mol% (which was 0.98 ± 0.17), where the cell growth is slightly promoted instead LA production. The relative LA to biomass yield changes between 1 and 1.6 for dodecanol concentrations between 0 to 52.85 mol% (within of the organic phase TiOA/dodecanol/dodecane). A maximum LA to biomass yield of 6.6 is reached for a dodecanol concentration of 94.66 mol%, where the LA production was promoted instead cell growth. Relative LA to biomass yields higher than the unit is due to biomass growth is always lower than the LA produced, except for a dodecanol concentration of 0 mol%.

However, a high LA to biomass yield does not mean a high LA productivity as compared to the control fermentation. At the highest relative LA to biomass yield, it is obtained the lowest LA and biomass concentrations (Figure 2) due to the high toxicity level.

3.2.4 Conclusions

Molecular toxicity on the *Lactobacillus casei* ATCC 393 and liquid-liquid equilibria within aqueous lactic acid, for mixtures of TiOA/dodecane, TiOA/dodecanol, both at 10 vol% (5.4 mol%) of TiOA, and TiOA/dodecanol/dodecane at a TiOA concentration of 10 vol% (5.4 to 5.34 mol%) and dodecanol concentrations of 0, 10.62, 21.22, 31.79, 42.33, 52.85 and 94.66 mol% were measured.

Both, the distribution coefficient and the chemical equilibrium constant are higher for the mixture TiOA/dodecanol than for the mixture TiOA/dodecane. However, the mixture TiOA/dodecanol was toxic on the bacteria, while the mixture TiOA/dodecane was non-toxic on the bacteria.

For the systems containing TiOA/dodecane/dodecanol in the organic phase, at several ratios dodecanol to dodecane, the distribution coefficient and the chemical equilibrium constant (K_E) increase as dodecanol concentration rises. However, the organic mixture at a dodecanol concentration of 10.62 mol% is non-toxic on the bacteria, while at dodecanol concentrations from 21.22 to 52.85 mol% were medium toxicity.

Cell growth is promoted instead of glucose consumption and LA production when the culture media is saturated with TiOA/dodecane. Glucose consumption and LA production are promoted instead cell growth when the culture media is saturated with TiOA/dodecanol. Even, the last behavior occurs for all organic phases with dodecanol, regardless of the presence of dodecane in these organic mixtures.

The highest value of K_E is achieved for the mixture TiOA/dodecanol, but the organic mixture is toxic on the bacteria. The values of K_E are quite similar (around 150 $L \cdot mol^{-1}$) at dodecanol concentrations of 31.78, 42.33 and 52.85 mol%, where the organic mixtures are medium toxicity. The mixtures of TiOA/dodecanol/dodecane at dodecanol concentrations between of 31.79 and 42.33 mol% have a good compromise between a high value of K_E and a relatively low molecular toxicity.

NOTATION

a_i	Fitted parameters in eq 3
K_1	Fitted parameter eq 4 [$L \cdot mol^{-1}$]
K_2	Fitted parameter eq 4 [$L \cdot mol^{-1}$]

K_D	Distribution coefficient
K_E	Chemical equilibrium constant [L·mol^{-1}]
DOH	Dodecanol
HPLC	High performance liquid chromatography
LA	Lactic acid
LA-TiOA	The complex between lactic acid and tri-iso-octylamine
LLE	Liquid-liquid equilibria

Subscripts

aq	Aqueous phase
org	Organic phase

3.2.5 References

[1] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[2] M. Singhvi, T. Zendo, K. Sonomoto, Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects, Appl. Microbiol. Biotechnol. 102 (2018) 5911–5924. doi:10.1007/s00253-018-9092-4.

[3] P. Pal, J. Sikder, S. Roy, L. Giorno, Process intensification in lactic acid production: A review of membrane based processes, Chem. Eng. Process. Process Intensif. 48 (2009) 1549–1559.

[4] A. Komesu, M.R. Wolf Maciel, R. Maciel Filho, Separation and Purification Technologies for Lactic Acid – A Brief Review, BioResources. 12 (2017) 6885–6901. doi:10.15376/biores.12.3.6885-6901.

[5] T. Ghaffar, M. Irshad, Z. Anwar, T. Aqil, Z. Zulifqar, A. Tariq, M. Kamran, N. Ehsan, S. Mehmood, Recent trends in lactic acid biotechnology: A brief review on production to purification, J. Radiat. Res. Appl. Sci. 7 (2014) 222–229. doi:10.1016/j.jrras.2014.03.002.

[6] J. Vijayakumar, R. Aravindand, T. Viruthagiri, Recent Trends in the Production, Purification and Application of Lactic Acid, Chem. Biochem. Eng. Q. 22 (2008) 245–264. https://hrcak.srce.hr/24811.

[7] M. Othman, A.B. Ariff, H. Wasoh, M.R. Kapri, M. Halim, Strategies for improving production performance of probiotic Pediococcus acidilactici viable cell by overcoming lactic acid inhibition, AMB Express. 7 (2017). doi:10.1186/s13568-017-0519-6.

[8] M. Othman, A.B. Ariff, L. Rios-Solis, M. Halim, Extractive fermentation of lactic acid in lactic acid bacteria cultivation: A review, Front. Microbiol. 8 (2017) 1–7. doi:10.3389/fmicb.2017.02285.

[9] A. Demirci, J.C. Cotton, A.L. Pometto, K.R. Harkins, P.N. Hinz, Resistance of Lactobacillus casei in plastic-composite-support biofilm reactors during liquid membrane extraction and optimization of the lactic acid extraction system., Biotechnol. Bioeng. 83 (2003) 749–59.

[10] N. Phanthumchinda, S. Thitiprasert, S. Tanasupawat, S. Assabumrungrat, N. Thongchul, Process and cost modeling of lactic acid recovery from fermentation broths by membrane-based process, Process Biochem. 68 (2018) 205–213. doi:10.1016/j.procbio.2018.02.013.

[11] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702.

[12] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria for Trioctylamine/1-Dodecanol/Lactic Acid/Water System at 306.1, 310.1 and 316.1 K: Experimental Data and Prediction, J. Chem. Eng. Data. 61 (2016) 2269–2276.

[13] A. Krzyzaniak, B. Schuur, A.B. De Haan, Equilibrium studies on lactic acid extraction with N,N-didodecylpyridin-4-amine (DDAP) extractant, Chem. Eng. Sci. 109 (2014) 236–243.

[14] A. Krzyzaniak, M. Leeman, F. Vossebeld, T.J. Visser, B. Schuur, A.B. De Haan, Novel extractants for the recovery of fermentation derived lactic acid, Sep. Purif. Technol. 111 (2013) 82–89.

[15] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[16] R. Juang, R. Huang, Equilibrium studies on reactive extraction of lactic acid with an amine extractant, Chem. Eng. J. 65 (1997) 47–53.

[17] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications,

3rd ed., Elsevier, Amsterdan, 2003.

[18] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[19] A.D. Pérez, B. Van der Bruggen, J. Fontalvo, Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation, Chem. Eng. Process. - Process Intensif. 134 (2018) 20–27.

[20] A. Kumar, A. Thakur, P.S. Panesar, Lactic acid extraction using environmentally benign Green emulsion ionic liquid membrane, J. Clean. Prod. 181 (2018) 574–583. doi:10.1016/j.jclepro.2018.01.263.

[21] F. Garavand, S.H. Razavi, I. Cacciotti, Synchronized extraction and purification of L-lactic acid from fermentation broth by emulsion liquid membrane technique, J. Dispers. Sci. Technol. 39 (2018) 1291–1299. doi:10.1080/01932691.2017.1396225.

[22] N. Rastogi, B.S. Chanukya, Supported Liquid Membrane Composed of Tertiary or/and Quaternary Amine for the Extraction of Lactic Acid, Int. J. Membr. Sci. Technol. 2 (2015) 19–28. doi:10.15379/2410-1869.2015.02.02.03.

[23] A. Manzak, O. Tutkun, The extraction of lactic acid by emulsion type of liquid membranes using alamine 336 in escaid 100, Can. J. Chem. Eng. 89 (2011) 1458–1463. doi:10.1002/cjce.20501.

[24] R. Juang, S. Lee, R. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239.

[25] R. Chen, Y.Y. Lee, Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass, Appl. Biochem. Biotechnol. 63–65 (1997) 435–448. doi:10.1007/BF02920444.

[26] R. Juang, R. Huang, Separation of citric and lactic acids in aqueous solutions by solvent extraction and liquid membrane processes, J. Memb. Sci. 136 (1997) 89–99.

[27] C. Schöller, J.B. Chaudhuri, D.L. Pyle, Emulsion liquid membrane extraction of lactic acid from aqueous solutions and fermentation broth, Biotechnol. Bioeng. 42 (1993) 50–58. doi:10.1002/bit.260420108.

[28] Z. Gu, B.A. Glatz, C.E. Glatz, Propionic acid production by extractive fermentation. I.

Solvent considerations, Biotechnol. Bioeng. 57 (1998) 454–461. doi:10.1002/(SICI)1097-0290(19980220)57:4<454::AID-BIT9>3.0.CO;2-L.

[29] J. Marták, E. Sabolová, Š. Schlosser, M. Rosenberg, L. Kristofíková, Toxicity of organic solvents used in situ in fermentation of lactic acid by Rhizopus arrhizus, Biotechnol. Tech. 11 (1997) 71–75. doi:10.1023/A:1018408220465.

[30] B. Choudhury, A. Basha, T. Swaminathan, Study of Lactic Acid Extraction with Higher Molecular Weight Aliphatic Amines, J. Chem. Technol. Biotechnol. 72 (1998) 111–116.

[31] A. Demirci, A.L. Pometto, K.R. Harkins, Rapid screening of solvents and carrier compounds for lactic acid recovery by emulsion liquid extraction and toxicity on Lactobacillus casei (ATCC 11443), Bioseparation. 7 (1999) 297–308.

[32] V.M. Yabannavar, D.I.C. Wang, Strategies for reducing solvent toxicity in extractive fermentations, Biotechnol. Bioeng. 37 (1991) 716–722. doi:10.1002/bit.260370805.

[33] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using Lactobacillus casei ATCC 393, Dyna. 85 (2018) 360–366. doi:10.15446/dyna.v85n207.72374.

[34] S. Torres, A. Pandey, G.R. Castro, Organic solvent adaptation of Gram positive bacteria: Applications and biotechnological potentials, Biotechnol. Adv. 29 (2011) 442–452. doi:10.1016/j.biotechadv.2011.04.002.

[35] H. Kusumawardhani, R. Hosseini, J.H. de Winde, Solvent Tolerance in Bacteria: Fulfilling the Promise of the Biotech Era?, Trends Biotechnol. 36 (2018) 1025–1039. doi:10.1016/j.tibtech.2018.04.007.

[36] A. Segura, L. Molina, S. Fillet, T. Krell, P. Bernal, J. Muñoz-Rojas, J.L. Ramos, Solvent tolerance in Gram-negative bacteria, Curr. Opin. Biotechnol. 23 (2012) 415–421. doi:10.1016/j.copbio.2011.11.015.

[37] C.S. López-Garzón, A.J.J. Straathof, Recovery of carboxylic acids produced by fermentation, Biotechnol. Adv. 32 (2014) 873–904. doi:10.1016/j.biotechadv.2014.04.002.

[38] N. Tik, E. Bayraktar, Ül. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, J. Chem. Technol. Biotechnol. 76 (2001) 764–768.

[39] W. Qin, Z. Li, Y. Dai, Extraction of Monocarboxylic Acids with Trioctylamine: Equilibria

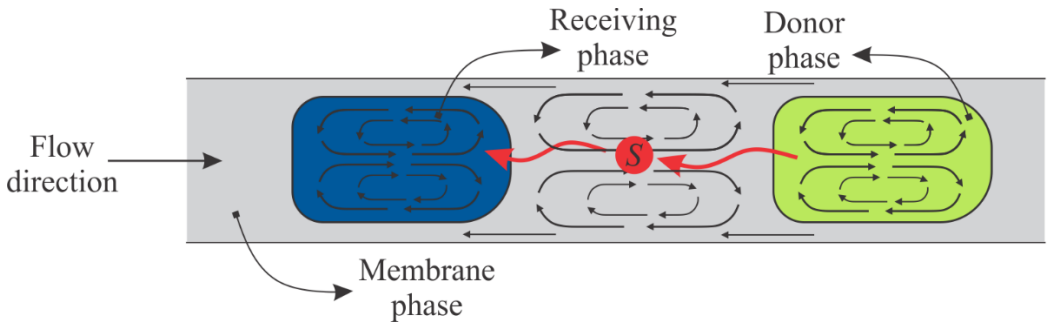
and Correlation of Apparent Reactive Equilibrium Constant, Ind. Eng. Chem. Res. 42 (2003) 6196–6204.

[40] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria of Lactic Acid/Water Solutions in Tri-iso-octylamine/Dodecane/1-Dodecanol at 306.1, 310.1, and 316.1 K. Experimental Data and Prediction, J. Chem. Eng. Data. submited (2019) acs.jced.8b00794. doi:10.1021/acs.jced.8b00794.

[41] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[42] V.M. Yabannavar, D.I.C. Wang, Bioreactor System with Solvent Extraction for Organic Acid Production, Ann. N. Y. Acad. Sci. 506 (1987) 523–535. doi:10.1111/j.1749-6632.1987.tb23847.x.

4. Chapter 4: Liquid membrane in Taylor flow



4.1 A new concept of liquid membranes in Taylor flow: performance for lactic acid removal[5]

Abstract

A liquid membrane in Taylor flow regime is a novel alternative kind of contact in three phase flow for liquid membranes that preserves the advantages of conventional emulsion liquid membranes while overcomes the stability problems of emulsion systems. As a proof of concept, this work presents experimental results of a liquid membrane in Taylor flow for lactic acid removal. Several operating conditions, such as injection times, delay times and flow of the membrane phase were tested for a channel length and inner diameter of 348.8 cm and 2.5 mm, respectively. The lactic acid removal is mainly affected by the driving force of lactic acid concentrations between donor droplets and the membrane interface, and the space-time. Thus, the lactic acid removal process through the liquid membrane in Taylor flow is enhanced at low injection times and high droplet velocity considering that enough space-time is provided. This technology results promising as an alternative to conventional liquid membranes and the intensification of chemical and fermentative processes.

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4.1.1 Introduction

Liquid membranes

In a liquid membrane (LM) process three fluid phases are continuously in contact [1]: membrane phase (*M*), donor phase (*D*) and receiving phase (*R*). Membrane phase is an immiscible semipermeable barrier which separates the donor phase from the receiving phase [1,2]. The donor phase contains the solute that is transported from the donor to the receiving phase through the membrane phase. Transport process in liquid membranes involves both liquid-liquid extraction (LLE) and membrane separation in a single device [1]. Usually, the membrane phase is organic [1,2] and comprises a solvent of the LLE process which can include a carrier. When the carrier is within the membrane phase, it reacts spontaneously, rapidly and reversibly with the solute of the donor phase forming a complex which is transported from the *D*/*M* interphase to *M*/*R* interphase (facilitated transport), and here, the solute is released to the receiving phase [1].

LMs is a perstraction process that is classified as bulk (BLM), supported (SLM) and emulsion (ELM) liquid membranes according to its configuration [1]. BLMs are commonly used for mass transport and kinetic studies at lab-scale because it is limited by its low specific interface area [2]. SLMs and ELM have potential on applications in industrial scale because they provide large interfacial areas, extraction and stripping are in one stage, simple operation and it is possible to process high quantities of compounds (from donor phase) using small volumes of the membrane phase [1,3]. However, SLMs and ELMs have some stability problems that are limiting their use in industry. On the one hand, during the separation process through a SLM there are losses of the membrane phase components that lead to flux decreasing and the support has to be refilled with the membrane phase [1,2,4]. On the other hand, ELMs require mixing, decantation, and the addition of surfactants to keep stable the double emulsion and, in consequence, drops do not easily break-up to recover the receiving phase [1].

Taylor flow

Taylor flow (or slug flow) is a type of two-phase flow regime, where a liquid phase flows continuously (continuous phase) within a tube or channel, and there is a periodic occurrence of elongated droplets or bubbles (dispersed phase) within the same channel. Besides geometrical and physicochemical parameters, Taylor flow depends on the flow rate ratio between the continuous and the dispersed phases [5]. The segment of liquid that travels between the droplets (or bubbles) is called slug [5,6]. Taylor flow is characterized by the formation of a toroidal vortex within the slug [5,7,8] and into the droplets [5,9]. In this kind of two-phase flow, heat and mass transfer between the

phases is enhanced due to the presence of the internal circulations that helps to renew the interfaces. Also, there is a high specific interfacial area and a high mass and heat transfer in a liquid film, next to the tube wall, that surrounds the dispersed drops [10,11].

Taylor flow regime can be predicted using the capillary number (*Ca*) that relates the viscous with interfacial tension forces [12]. The Capillary number is defined as $Ca = U_d^* \mu/\sigma$, where U_d is the mean droplet velocity, μ is the viscosity of the continuous phase, and σ is the interfacial tension between the phases. The values of *Ca* in Taylor flow are low because the interfacial tension force dominates over the viscous force. Additionally, *Ca* can be used to predict the hydrodynamic conditions of the system such as the thickness of the liquid film [13], the vortex formation and the bubbles or droplet shape [5,9].

Application of Taylor flow on liquid-liquid extraction has attracted the attention of several researchers due to the enhanced characteristics in mass transfer of this regime flow. Current studies are focused on the influence of the geometry of the channel on mass transfer [14], on the influence of the operating conditions such as flow rate ratio (continuous to dispersed phase) and slug length on mass transfer performance [15], on incorporation of twisted mixers to the channels [16], and on the configuration channel design [17], among others.

The liquid membrane in Taylor flow regime

In this work, an alternative kind of contact among phases of a liquid membrane has been developed by extending the Taylor flow regimen to a three-phase system [18,19]. In this membrane technology, called liquid membrane in Taylor flow regime (LMTF), the membrane phase is used as a continuous phase (slugs), and the donor and receiving phases are dispersed aqueous phases (droplets). All these phases flow within a channel. The solute (*S*) is transferred from the donor phase to the membrane phase and, from here, to the receiving phase (Figure 1).

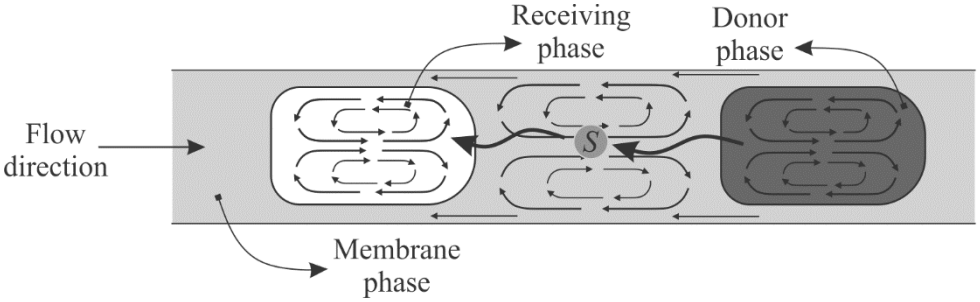

Figure 1. Scheme for solute (S) transport in a liquid membrane in Taylor flow regime [18].

As a proof of concept of the LMTF, this work presents experimental results of lactic acid removal at several operating conditions such as volumetric membrane phase flow, donor and receiving volumetric injection flow, injection time of the dispersed phases and delay time.

Description of a liquid membrane in Taylor flow regime

The LMTF operates by injecting a single droplet or several droplets of each aqueous phase. The injection of the dispersed phases (donor and receiving) is carried out by cycles while the membrane phase flows continuously through the channel. Each injection cycle follows the next four steps: First, the donor phase is injected during an injection time of the donor phase (t_{inj}^{D}, the elapsed time for constant injection flow of the donor phase). Then, the injection of the donor phase is stopped and a delay time (t_{del}, the elapsed time from the end of the injection of the donor phase to the start of the injection of the receiving phase) elapses. Afterward, the receiving phase starts to inject during an injection time of receiving phase (t_{inj}^{D}, the elapsed time for constant injection flow of the receiving phase), and in the fourth step, the receiving phase injection is stopped and a delay time (t_{del}) elapses again.

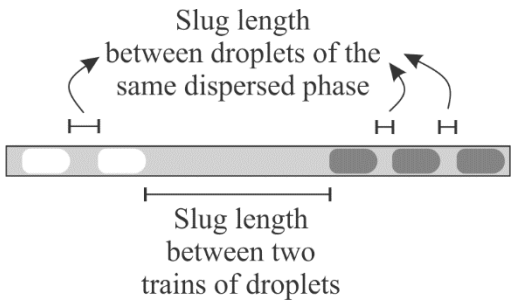

Figure 2. Scheme of the slug lengths that are formed in the LMTF system [18].

A train of droplets of the respective phase is formed during the injection of the donor or receiving phases. Two kinds of slugs, with their respective lengths, are formed during each injection cycle (Figure 2). There is a slug length between the back cap of the last droplet of a train of droplets of the donor phase and the front cap of the first droplet of the subsequent train of droplets of the receiving phase. Another slug is located within the train of droplets of the same phase (donor or receiving), and its length is given between the back cap of a droplet and the front cap of the subsequent droplet of the same phase.

The velocity of each phase could change and depends on the flow of the membrane phase and the injection flow of each dispersed phase. During delay times, the only phase that is injected is the

membrane phase while during injection times both dispersed (donor or receiving) and membrane phases are flowing. Therefore, the total flow during the injection time is higher than the total flow during the delay time, causing changes in velocity for the continuous phase (membrane) and the droplets (donor and receiving).

Lactic acid extraction

Long-chain aliphatic amines have proved to be efficient for organic acid extraction from aqueous solutions [20–25] where the tertiary amines combined with other organic substances (diluents) have been the most widely used. Diluents are used to improve the physical properties of the organic phase such as density, viscosity, interfacial tension, and the extractive capacity [20,24,26]. Trioctylamine (TOA) and tri-iso-octylamine (TiOA) are common tertiary amines used for organic acid removal from aqueous solutions [27–32]. The tertiary amine reacts with the organic acid in the aqueous/organic interface producing an amine-organic acid complex which favored the extraction process of the organic acid [33,34]. Tertiary amines provide high extraction availability, low water solubility and high selectivity [28,35–38].

Currently, there are several studies for LA extraction where potential extractants have been tested. For instance, tertiary amines and other extractants have been modified by adding functionalized silica compounds that provide higher capacity for extraction of the lactic acid [39]. The ionic liquids are another alternative for organic acid extraction that has been tested for LA removal providing high distribution coefficients [40,41]. N,N-didodecylpyridin-4-amine (DDAP) extractant is another potential extractant tested for LA removal that reaches LA extraction till 99% [42].

In perstraction processes, while the solute is transported to the membrane phase it is continuously removed from it by the receiving phase [2]. Hence, it is not necessary to use the extractant with the highest distribution coefficient for the organic acid because perstraction processes are not limited by the thermodynamic liquid-liquid equilibria, unlike the liquid-liquid extraction processes. However, if the perstraction process is used for in-situ removal of the organic acid from the fermentation broth, the toxicity of the membrane phase must be taken into account for selecting of a membrane phase to achieve a good compromise between a high value of distribution coefficient and a relatively low toxicity.

In previous works, both the liquid-liquid equilibria of potential membrane phases for LA removal and the molecular toxicity of these potential membrane phases on the *Lactobacillus casei* ATCC 393 (lactic acid bacteria) have been tested [32,43–45]. In this works, a membrane phase composed by

TiOA, 1-dodecanol, and n-dodecane has been used which provides a relative low molecular toxicity on the *Lactobacillus casei* ATCC 393 and a relative high LA extraction capacity [44].

4.1.2 Experimental

Materials

Tri-iso-octylamine (assay 95 %), n-dodecane (assay 99 %), 1-dodecanol (assay 98 %), sulfuric acid (assay 95-97 %) and sodium carbonate anhydrous (assay 99.5 %) were supplied by Merck Millipore. L(+)-lactic acid was supplied by Panreac Química S.A.U. (assay 88.0-92.0 %). The lactic acid, according to the supplier, contains a maximum concentration of metals of 0.001 wt%. However, HPLC measurements do not show additional peaks besides lactic acid. The purity of lactic acid was assessed by titration with sodium hydroxide of Carlo Herba (assay ≥ 97.0 %) using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). Type I water was used for all aqueous solutions (Barnstead™ Nanopure™).

Preparation of the phases

The membrane phase is composed by a mixture of tri-iso-octylamine (TiOA), 1-dodecanol and n-dodecane at 10, 40 and 50 vol%, respectively, where the amine is the carrier, dodecanol an active diluent and dodecane an inert diluent [45]. The donor phase was an aqueous solution of lactic acid at 10 g/L prepared from a stock solution of lactic acid at 150 g/L previously heated at 90 °C under total reflux between 8-10 hours for dimer hydrolysis [32,46,47] and it was quantified by titration using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). The receiving phase was an aqueous solution of sodium carbonate at 2.5 g/L.

Liquid membrane transport between the phases

The carrier, TiOA, within the membrane phase reacts with lactic acid (LA, which is the solute) of the donor phase in the *D*/*M* interphase to produce a LA-TiOA complex [45] in the side of the liquid film of the membrane phase where is located the reaction plane [48]. Also, free LA is solubilized into the membrane phase [32]. Both, LA and LA-TiOA complex are transported to the *M*/*R* interphase where an instantaneous acid-base reaction is carried out between sodium carbonate (of the receiving phase) and LA (Figure 3). Thus, the receiving phase contains sodium lactate and free LA.

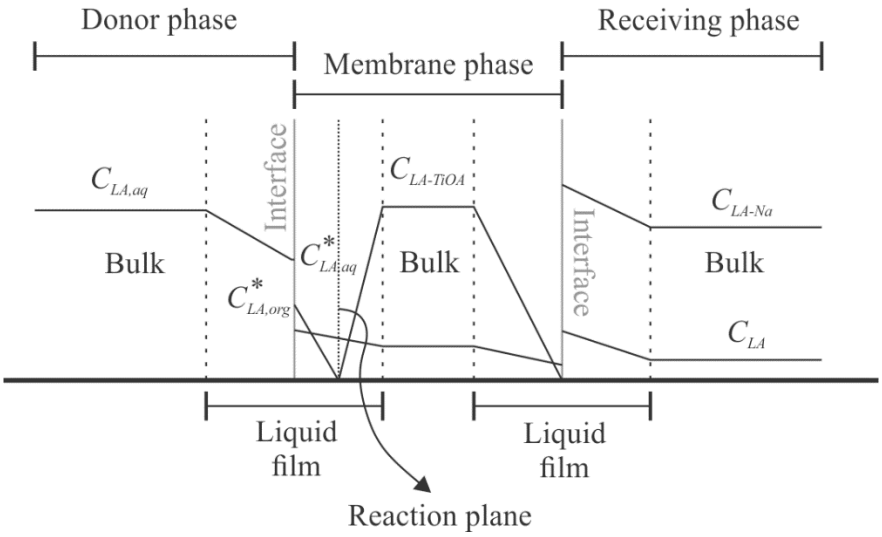

Figure 3. Lactic acid transport in a liquid membrane in Taylor flow (LMTF) regime with a membrane phase that contains a carrier (TiOA). *D*: Donor phase. *M*: Membrane phase. *R*: Receiving phase (where LA-Na is sodium lactate).

4.1.3 Experimental setup and calculations

The lactic acid fermentation are commonly carried out in the range of 35 to 45 °C, where the optimal temperature of the fermentation depends on the strain used [49–51]. Therefore, we previously carried out several LA fermentations by *Lactobacillus casei* ATCC 393 in this range of temperatures and the optimal fermentation temperature was 37 °C (not shown). Hence, the experiments for LA removal through the LMTF were carried out at 37 °C.

The experimental setup consists of a transparent polyurethane circular channel with an inner diameter of 2.5 mm and a length of 348.8 cm. This channel was coiled in a vertical length of 45 cm inside of a chamber with a controlled temperature of 37 ± 0.5 °C. In the bottom of the channel, two T-junctions were located at 4 cm each other to inject the donor and receiving phases (Figure 4). The donor phase (*D*) was injected by a syringe pump (Cole-Parmer® Touch Screen Infuse/Withdraw) setting the injection time, delay time and the flow rate. The receiving phase (*R*) was injected by a gear pump (Ismatec, Reglo-z) with a pump-head (Ismatec Z-186) controlling the injection and delay time by an Arduino Mega interface coupled to a solenoid valve (STNC® - DC 24V). Donor, receiving and membrane phases outflowed at the top channel to a settler with two compartments (lab-made of polytetrafluoroethylene, PTFE) to independently split the donor and receiving phases from the membrane phase. When the train of donor droplets with membrane slugs are near to leave the channel, the outside of the channel is located in one of the compartments of the settler. By

knowing the injection times of each phase it is possible to predict the time when the corresponding phase is going to arrive at the top of the channel and so the liquid is driven to the corresponding compartment of the settler (donor or receiving). In each compartment, the corresponding aqueous phase is separated from the membrane phase and the membrane phase is recirculated to the inlet of the LMTF system.

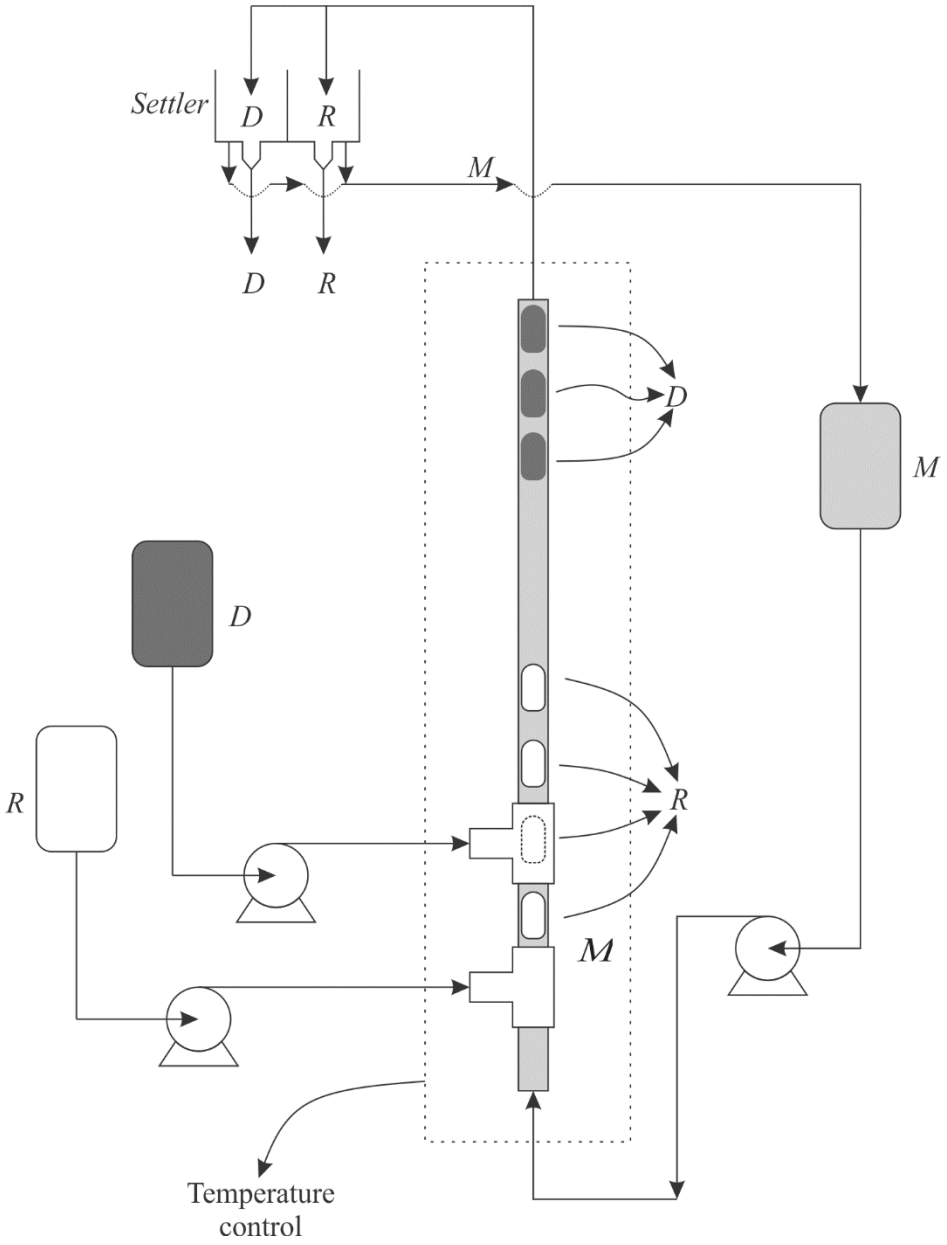

Figure 4. Schematic experimental set up to carry out the performance test of the liquid membrane in Taylor flow for lactic acid removal [18].

A HPLC pump (Waters 501) was used to continuously feed the membrane phase (*M*) at the bottom of the channel and, after decantation at the top of the channel, it was recycled. Samples of the donor and receiving phases were taken from each container in the settler during an experiment until their LA concentrations were constant (30 to 50 min). Both aqueous phases were constantly purged from their respective containers in the settler. Lactic acid concentrations were measured by HPLC with an ORH-801 column (Chrom Tech), an aqueous solution of 0.01 N of sulfuric acid for the mobile phase, and a RI detector at 45 °C [32].

Several flow rates of the membrane phase, and of the dispersed phases (donor and receiving) were tested at several injections (t_{inj}) and delay times (t_{del}). For every single experiment, the injection times of donor and receiving phases were the same. The operating conditions for each experiment are shown in Table 1.

Table 1. Experimental conditions for lactic acid removal with a LMTF system.

Donor flow rate (Q_D) of 2 mL/min. Capillary numbers (for *D*/*M* and *R*/*M*) were calculated as it is shown in [52].

Membrane flow rate, Q_M (mL/min)	Receiving flow rate, Q_R (mL/min)	Injection time, t_{inj} (s)	Delay time, t_{del} (s)	$Ca_{mix,D}$	$Ca_{mix,R}$
4.5	1.16	6	6	0.0030	0.0028
4.5	1.16	12	12	0.0030	0.0028
4.5	1.16	16	16	0.0030	0.0028
6.5	1.03	6	6	0.0042	0.0040
6.5	1.03	12	12	0.0042	0.0040
6.5	1.03	16	16	0.0042	0.0040
8.5	0.73	6	6	0.0054	0.0052
8.5	0.73	12	12	0.0054	0.0052
8.5	0.73	16	16	0.0054	0.0052
9.9	0.70	6	6	0.0063	0.0061
9.9	0.70	12	12	0.0063	0.0061

The total flow when donor ($Q_{D,Tot}$) and receiving ($Q_{R,Tot}$) phases are injecting is calculated taking into account the flow of the continuous phase (Q_M) as is shown below:

$$Q_{D,Tot} = Q_M + Q_D \tag{1}$$

$$Q_{R,Tot} = Q_M + Q_R \quad (2)$$

The average velocity of each phase was calculated as follows:

$$U_D = Q_{D,Tot} / A \quad (3)$$

$$U_R = Q_{R,Tot} / A \quad (4)$$

where *A* is the transversal area of the channel. The space-time of the dispersed phases was calculated taking into account the time from the injection point (bottom of the channel) to the outside of the channel (this distance corresponds to the channel length, L_C).

$$\tau_D = L_C / U_D \quad (5)$$

$$\tau_R = L_C / U_R \quad (6)$$

The degree of LA removed in the LMTF (*Ra*) is defined as the ratio between the amount of LA removed from donor phase (R_{LA}) and the total amount of LA acid that theoretically can be accepted in the receiving phase ($LA_{complex}$+ LA_{free}), equation (8). LA in the receiving phase is accepted as sodium lactate ($LA_{complex}$) and as free LA acid. The maximum theoretical amount of LA as sodium lactate ($LA_{complex}$) depends on the stoichiometry of equation (7), while the amount of free LA depends on the final amount of LA in the donor phase (LA_{free}).

$$2\ CH_3\text{-}CH(OH)\text{-}COOH + Na_2CO_3 \rightarrow 2\ CH_3\text{-}CH(OH)\text{-}COO^-Na^+ + H_2CO_3 \quad (7)$$

$$Ra = \frac{R_{LA}}{LA_{complex} + LA_{free}} \quad (8)$$

In the receiving phase, the sodium lactate is first formed till the sodium carbonate is exhausted, and the additional LA is transferred to the receiving phase as a free LA. At the top of the channel the maximum theoretical amount of free LA (LA_{free}) in the receiving phase is close to the concentration of LA in the donor phase. Thus, the final concentration in the donor phase was used as the maximum theoretical amount of free LA (LA_{free}) in the receiving phase in equation (8). *Ra* is related to the amount of LA transported but it is limited by thermodynamics. Thus, if the LA activity in the donor and receiving phases are equal at some point within the channel the LA mass transfer ceases. However, in this paper, as a shortcut, instead LA activities we have used LA concentrations and thus

the LA activity coefficient is the unity for both donor and receiving phases. Thus, the maximum value of *Ra* can be slightly higher than one because actually LA activity coefficients are slightly different from one which corresponds to non-ideal aqueous phases. On the other hand, *Ra* also includes the flow rates of donor and receiving phases (nomenclature section).

4.1.4 Results and discussion

Flow characterization

Figures 5-7 show the hydrodynamic behavior of the LMTF system regarding droplet velocity, slug length (between donor and receiving phases) and injected volume of the dispersed phases at the tested experimental conditions presented in Table 1. Figure 5a shows that the velocity of the donor droplets increases as the membrane flow rate rises, regardless of both delay and injection times. For all operating conditions, the velocity of the droplets of the receiving phase was lower than the velocity of the droplets of the donor phase (Figure 5b), due to an also lower flow rate of the receiving phase. In the LMTF system can be expected a difference of velocities between the donor droplets and receiving droplets because their physical properties differ as density, viscosity, and interfacial tension. The density of the aqueous phases was measured experimentally at 25 °C, achieving similar values of 1.0013 and 1.0239 kg/m^3 for donor and receiving phases, respectively. In contrast, experimental values for viscosity are different at 25 °C, being 1.17 and 0.7669 mPa·s for donor and receiving phases, respectively. Thus, the donor phase has higher shear stress that allows the droplet to travel faster with an increase of the membrane flow rate than the receiving phase in co-current flow. Goldsmith and Mason [8] have shown that a high viscosity difference produces a high droplet velocity in a liquid-liquid Taylor flow system, and in an additional study, it was observed that more viscous droplets move faster than less viscous ones [53]. On the other hand, the interfacial tension between the membrane phase and the respective dispersed phase (donor or receiving) also have a direct effect on the capillary number and the shape of the droplets [5]. Both, interfacial tension and viscosity influence the hydrodynamic behavior of the phases in Taylor flow regime. The ratio between these properties (σ/μ) is called interfacial velocity, and it has been related to the stability of droplet formation in Taylor flow [8,54,55]. The interfacial tension of receiving and donor phases were not experimentally measured in this work. According to previous CFD simulation that we performed (not shown) it was observed that at the low viscosity ratios (dispersed/membrane) of these experiments, lower than the unit, the influence of the interfacial tension on the interfacial velocity is negligible. Also, the interfacial velocity around the droplets in the organic film is higher at low viscosity ratios than at high viscosity ratios.

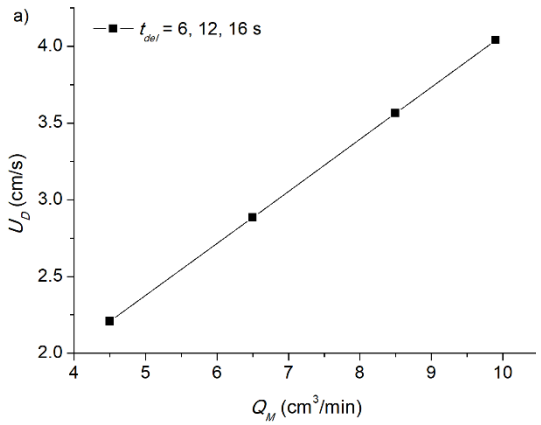

Figure 5. a) Velocity of the donor droplets at several membrane flow rates.

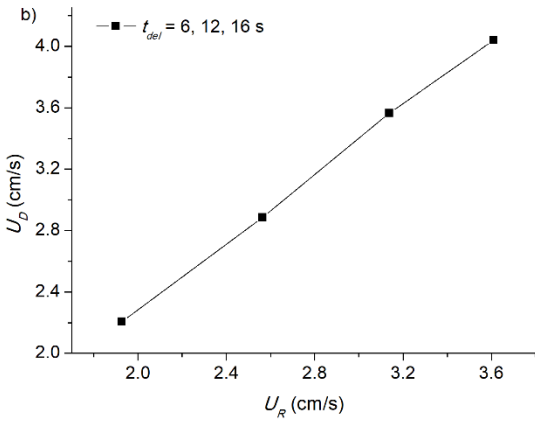

Figure 5. b) Velocity of donor droplets at each corresponding velocity of receiving droplets.

For the membrane phase, high flow rates produce high injected volumes and whereby the slug length is also high (Figure 6). On the other hand, for a fixed flow rate of the membrane phase, a high delay time involves a high injected volume of the membrane phase as well, risen the slug length and producing a higher slope in Figure 6 as delay time rises.

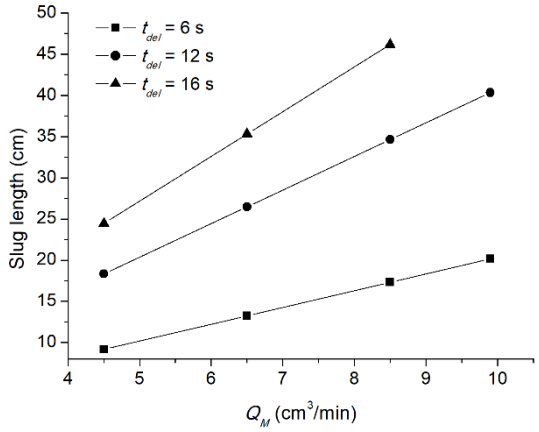

Figure 6. Slug length between the train of droplets of the donor phase and the subsequent train of droplets of the receiving phase as a function of the membrane flow rate for three delay times. Use Figure 5 to read donor phase and receiving phase velocities at each membrane flow rate.

The volume of the receiving phase per injection cycle increases as the injection time rises for constant membrane and donor flow rates (Figure 7). On the contrary, the volume of the receiving phase decreases as the membrane flow rate rises for a constant delay time. For injection times of 6, 12 and 16 s, the injected volumes of the donor phase were 0.2, 0.4 and 0.54 cm^3, respectively. For a

constant membrane flow rate, the injected volume of the donor phase was higher, between 41 and 63 %, than the injected volume of the receiving phase.

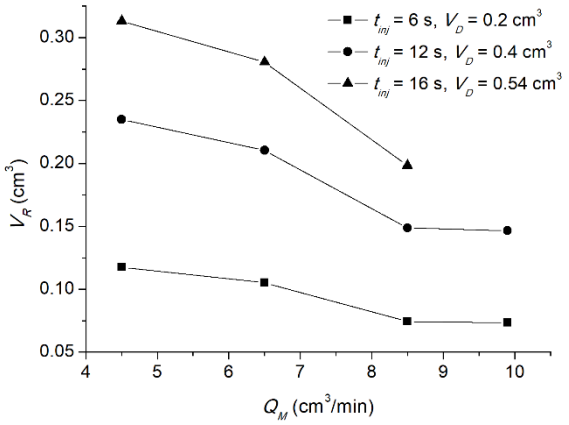

Figure 7. Injected volumes of the receiving phase at several flow rates of the membrane phase and injection times. Use Figure 5 to read donor phase and receiving phase velocities at each membrane flow rate.

Performance of the LMTF

The behavior of LA transported from donor to receiving phase is shown as a function of the donor droplet velocity, and the slug length in Figures 8 and 9, respectively. The degree of LA removal increases as the velocity of the donor droplet rises (Figure 8). At high donor droplet velocities, the LA concentration at the interphase renews faster than at low velocities [56,57]. Also, the higher the velocity of the donor droplets is, the higher the mixing caused by the vortex [55] will be, reducing stagnant zones within the slugs. Additionally, it is observed that *Ra* is almost constant at high velocities of donor droplets, where the LA mass transport is at its maximum.

The removal process of LA through the LMTF depends on two factors. The driving force of LA concentrations between donor droplets and the membrane interface (*D/M*), and the space-time (equations 7 and 8) of the droplets in the channel. High values of *Ra* are expected for high mass transfer rates and high space-times. At low droplet velocities, the mass transfer is low, but the space-time is high. At high droplet velocities the mass transfer is high, but the space-time low.

Since the space-time is inversely proportional to droplet velocity, for a constant channel length of 348.8.cm, the separation process achieves a maximum *Ra*, which occurs at a velocity of 3.5 cm/s (Figure 8). Thus, despite the high velocities, which provide high mixing for the LA transport, the space-time of the donor phase was not long enough to reach values of *Ra* close to one (except for a t_{inj} = 6s). Solute concentrations within the slug, between the donor droplets, are higher in short slugs

than within long ones which have been observed for conventional liquid-liquid Taylor flow [58]. Short slugs lengths (slugs between the donor droplets) are achieved with low droplet velocities which depend on the membrane flow rate (Figures 5a and 6). Therefore, the driving force between the LA concentration of the donor droplets and the membrane slugs increases as the donor droplet velocity rises (high slug lengths between donor droplets), and thus the value of *Ra* also increases (Figure 8). However, at high droplet velocities (>3.5 cm/s) the mass transfer of LA is limited by the space-time of the donor and receiving phases, and a limited *Ra* is achieved.

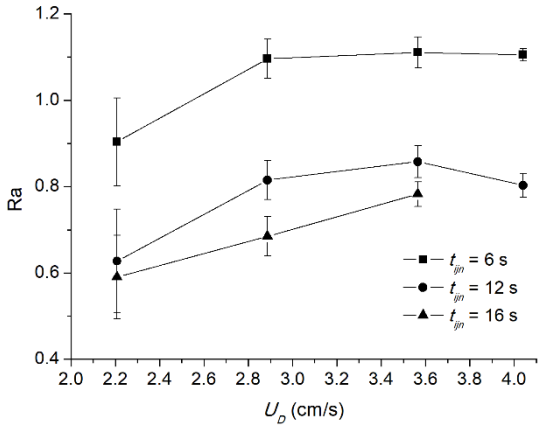

Figure 8. Degree of lactic acid removal at three injection times as a function of the donor droplet velocity.

According to the initial slope of *Ra* at each injection time (Figure 9), the slug length has a higher impact at low injection times than at high injection times. It is because, when low injection times (that provides low injection volumes) are used the amount of solute that can be transported to membrane phase is lower compared to high injection times, therefore, the driving force between donor phase and membrane phase is higher at low injection times generating a higher slope of *Ra* vs. slug length. On the other hand, *Ra* increases till achieving a maximum value at an optimal slug length, because beyond this point (which is the same points where the velocity of the donor droplets is beyond 3.5 cm/s in Figure 8) there is not long enough space-time for LA removal.

The abovementioned means that the degree of LA removal is enhanced with an increase of the slug length because a high driving force is induced. Nevertheless, exist a limiting value of the slug length, regarding the mass transfer [59], if it is provided a long enough space-time. When the slug length is too high, stagnant zones can appear between the vortex in the slug and the droplet which decrease the mass transfer between the droplet and the slug [7,60]. Consequently, there is an optimum slug length that produces a maximum *Ra* (Figure 9) for given injection and delay times.

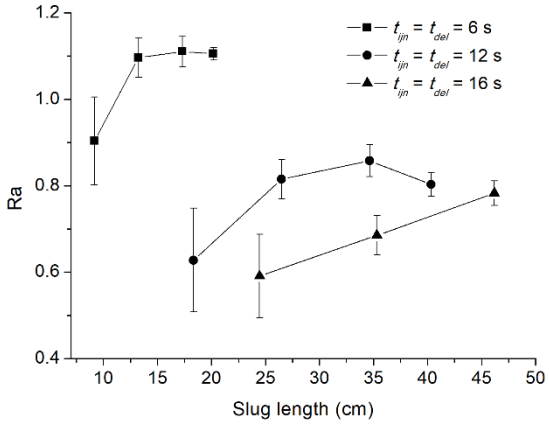

Figure 9. Effect of the slug length on the degree of lactic acid removal at different injection and delay times.

LA that is transported to the receiving phase as sodium lactate requires a lower space-time than LA that is received as a free acid because of the instantaneous reaction and high driving force in the former case [61]. Thus, *Ra* can also be enhanced if a low volume of receiving phase and a high concentration of sodium hydroxide is used.

Susanti *et al* [48] study the LA removal using an extraction process in Taylor flow without back-extraction. They found a 100% removal for a space-time of 90 s. In this study, a *Ra* of 1 is found for a residence time of 80 s where extraction and back-extraction occur simultaneously. However, between both studies there are differences in flow rates, channel length and diameter, organic phase composition and concentration, thus this comparison has to be performed with caution.

4.1.5 Conclusions

The lactic acid removal in a liquid membrane in Taylor flow regime (LMTF) was measured at several membrane flow rates, injection times of the dispersed phases and delay times. Also, the hydrodynamic behavior of the LMTF was measured as a function of these parameters. LMTF showed that is potentially useful for removal of LA acid from aqueous solutions.

The degree of LA removal (*Ra*) through the LMTF systems depends on the driving force of LA concentrations between donor droplets and the membrane interface (*D/M*), and the space-time of the phases within the channel. The LA removal among the phases is enhanced by low injection times and high velocity of dispersed phases, which produces long space-times and high mass transfer driving forces. Also, there is an optimal value of slug length for a given injection and delay times to achieve the maximum value of *Ra*.

The LMTF is a potential technology for industrial applications that preserves the advantages of conventional emulsion liquid membranes while overcomes the stability problems of emulsion systems. LMTF can be integrated to other systems and enables the intensification of chemical and fermentative processes

NOTATION

A	Transversal section area of the channel (cm^2)
C_{LA}	Lactic acid concentration (as free LA) at the receiving phase (g/L)
$C_{LA,aq}$	Lactic acid concentration fed at aqueous donor phase (g/L)
$C^*_{LA,aq}$	Lactic acid concentration in equilibria with membrane phase at the side of the aqueous phase (g/L)
$C_{LA\text{-}NA}$	Sodium lactate concentration at receiving phase (g/L)
$C_{LA,out}$	Final lactic acid concentration at the outside of the channel in the donor phase (g/L)
$C^*_{LA,org}$	Lactic acid concentration in equilibria with membrane phase at the side of the membrane phase (g/L)
$C_{LA\text{-}TiOA}$	Concentration of the LA-TiOA complex at membrane phase (g/L)
C_R	Sodium carbonate concentration in the receiving phase (g/L)
LA	Lactic acid
$LA_{complex} = V_R \cdot C_R \cdot R_{st} \cdot (MW_{LA}/MW_R)$	Maximum theoretical amount of lactic acid expected by equation (7) at receiving phase (g)
$LA_{free} = C_{LA,out} \cdot V_D$	Final amount of lactic acid in the donor phase (g)
LA-TiOA	Complex produced in the reaction between the lactic acid and the tri-iso-octylamine
L_C	Channel length (cm)
LMTF	Liquid membrane in Taylor flow
MW_{LA}	Lactic acid molecular weight
MW_R	Sodium carbonate molecular weight
Q_D	Flow rate of donor phase per injection (cm^3/s)
$Q_{D,Tot}$	Total flow rate when donor phase is injected (cm^3/s)
Q_M	Flow rate of membrane phase (cm^3/s)
Q_R	Flow rate of receiving phase per injection (cm^3/s)
$Q_{R,Tot}$	Total flow rate when receiving phase is injected (cm^3/s)
Ra	Degree of lactic acid removed in the LMTF system
$R_{LA} = Q_D \cdot t_{inj} \cdot (C_{LA,aq} - C_{LA,out})$	Amount of lactic acid removed from donor phase (g)
R_{st}	Stoichiometric ratio between lactic acid and sodium carbonate in equation (7)
$TiOA$	Tri-iso-octylamine

t_{del}	Delay time between injection of the two dispersed phases (s)
t_{inj}	Injection time of each dispersed phase (s)
U_D	Linear velocity of donor phase at injection point (cm/s)
U_R	Linear velocity of receiving phase at the injection point (cm/s)
V_D	Volume of donor phase per injection (cm^3)
V_R	Volume of receiving phase per injection (cm^3)
τ_D	Space-time of the donor droplets (s)
τ_R	Space-time of the receiving droplets (s)

4.1.6 References

[1] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[2] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications, 1st ed., Elsevier, Amsterdan, 2003.

[3] N.M. Kocherginsky, Q. Yang, L. Seelam, Recent advances in supported liquid membrane technology, Sep. Purif. Technol. 53 (2007) 171–177.

[4] A. Pérez de los Ríos, F.J. Hernández-Fernández, F. Tomás-Alonso, J.M. Palacios, G. Víllora, Stability studies of supported liquid membranes based on ionic liquids: Effect of surrounding phase nature, Desalination. 245 (2009) 776–782.

[5] T. Taha, Z.F. Cui, Hydrodynamics of slug flow inside capillaries, Chem. Eng. Sci. 59 (2004) 1181–1190.

[6] T.C. Thulasidas, M.A. Abraham, R.L. Cerro, Flow patterns in liquid slugs during bubble-train flow inside capillaries, Chem. Eng. Sci. 52 (1997) 2947–2962.

[7] G.I. Taylor, Deposition of a viscous fluid on a plane surface, J. Fluid Mech. 9 (1961) 218.

[8] H.L. Goldsmith, S.G. Mason, The flow suspensions through tubes. II. Single large bubbles, J. Coloid Sci. 18 (1963) 237–261.

[9] R. Gupta, S.S.Y. Leung, R. Manica, D.F. Fletcher, B.S. Haynes, Hydrodynamics of liquid–liquid Taylor flow in microchannels, Chem. Eng. Sci. 92 (2013) 180–189.

[10] D. Tsaoulidis, P. Angeli, Effect of channel size on liquid-liquid plug flow in small channels,

AIChE J. 62 (2016) 315–324.

[11] A. Ufer, M. Mendorf, A. Ghaini, D.W. Agar, Liquid-Liquid Slug Flow Capillary Microreactor, Chem. Eng. Technol. 34 (2011) 353–360.

[12] F.P. Bretherton, The motion of long bubbles in tubes, J. Fluid Mech. 10 (1961) 166–188.

[13] P. Aussillous, D. Quéré, Quick deposition of a fluid on the wall of a tube, Phys. Fluids. 12 (2000) 2367–2371.

[14] M. Sattari-Najafabadi, M. Nasr Esfahany, Z. Wu, B. Sundén, Hydrodynamics and mass transfer in liquid-liquid non-circular microchannels: Comparison of two aspect ratios and three junction structures, Chem. Eng. J. 322 (2017) 328–338.

[15] F. Kaske, S. Dick, S.A. Pajoohi, D.W. Agar, The influence of operating conditions on the mass transfer performance of a micro capillary contactor with liquid–liquid slug flow, Chem. Eng. Process. Process Intensif. 108 (2016) 10–16.

[16] O. Jafari, M. Rahimi, F.H. Kakavandi, Liquid-liquid extraction in twisted micromixers, Chem. Eng. Process. Process Intensif. 101 (2016) 33–40.

[17] P. Plouffe, D.M. Roberge, A. Macchi, Liquid-liquid flow regimes and mass transfer in various micro-reactors, Chem. Eng. J. 300 (2016) 9–19.

[18] J. Fontalvo, A.D. Pérez, Membrana Líquida y proceso para realizarlo, Colombian pending patent, Rad. 15-131023.

[19] A.D. Pérez, Desarrollo y evaluación de un sistema de membrana líquida en flujo de Taylor para la remoción de ácido láctico, Master dissertation, Universidad Nacional de Colombia, 2014.

[20] M. Marinova, J. Albet, J. Molinier, G. Kyuchoukov, Specific influence of the modifier (1-Decanol) on the extraction of tartaric acid by different extractants, Ind. Eng. Chem. Res. 44 (2005) 6534–6538.

[21] R. Canari, A.M. Eyal, Extraction of carboxylic acids by amine-based extractants: Apparent extractant basicity according to the pH of half-neutralization, Ind. Eng. Chem. Res. 42 (2003) 1285–1292.

[22] Z. Gu, B.A. Glatz, C.E. Glatz, Propionic acid production by extractive fermentation. I. Solvent considerations, Biotechnol. Bioeng. 57 (1998) 454–461.

[23] A.M. Eyal, R. Canari, pH Dependence of Carboxylic and Mineral Acid Extraction by Amine-Based Extractants: Effects of pKa, Amine Basicity, and Diluent Properties, Ind. Eng. Chem. Res. 34 (1995) 1789–1798.

[24] H. Ziegenfuß, G. Maurer, Distribution of acetic acid between water and organic solutions of tri-n-octylamine, Fluid Phase Equilib. 102 (1994) 211–255.

[25] S.T. Yang, S.A. White, S.T. Hsu, Extraction of Carboxylic Acids with Tertiary and Quaternary Amines: Effect of pH, Ind. Eng. Chem. Res. 30 (1991) 1335–1342.

[26] S. Pandey, S. Kumar, Reactive Extraction of Gallic Acid Using Aminic and Phosphoric Extractants Dissolved in Different Diluents: Effect of Solvent's Polarity and Column Design, Ind. Eng. Chem. Res. 57 (2018) 2976–2987.

[27] G. Malmary, J. Albet, A. Putranto, H. Hanine, J. Molinier, Measurement of partition coefficients of carboxylic acids between water and triisooctylamine dissolved in various diluents, J. Chem. Eng. Data. 43 (1998) 849–851.

[28] D.H. Han, W.H. Hong, Water-Enhanced Solubilities of Lactic Acid in Reactive Extraction Using Trioctylamine/Various Active Diluents Systems, Sep. Sci. Technol. 33 (1998) 271–281.

[29] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[30] G. Kyuchoukov, A. Labbaci, J. Albet, J. Molinier, Simultaneous Influence of Active and "Inert" Diluents on the Extraction of Lactic Acid by Means of Tri- n -octylamine (TOA) and Tri- iso -octylamine (TIOA), Ind. Eng. Chem. Res. 45 (2006) 503–510.

[31] W. Qin, Z. Li, Y. Dai, Extraction of Monocarboxylic Acids with Trioctylamine: Equilibria and Correlation of Apparent Reactive Equilibrium Constant, Ind. Eng. Chem. Res. 42 (2003) 6196–6204.

[32] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria for Trioctylamine/1-Dodecanol/Lactic Acid/Water System at 306.1, 310.1 and 316.1 K: Experimental Data and Prediction, J. Chem. Eng. Data. 61 (2016) 2269–2276.

[33] M. Hossain, Mass Transfer Investigation of Organic Acid Exatraction with Trioctylamine

and Aliquat 336 Dissolved in Various Solvents, in: Mass Transf. Multiph. Syst. Its Appl., InTech, 2011: pp. 367–388.

[34] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982.

[35] D. Yankov, J. Molinier, G. Kyuchoukov, J. Albet, G. Malmary, Improvement of the Lactic Acid Extraction . Extraction From Aqueous Solutions and Simulated Fermentation Broth by Means of Mixed Extractant and TOA , Partially Loaded with HCl, Chem. Biochem. Eng. Q. 19 (2005) 17–24.

[36] J.A. Tamada, A.S. Kertes, C.J. King, Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, Ind. Eng. Chem. Res. 29 (1990) 1319–1326.

[37] B. Choudhury, T. Swaminathan, Lactic acid extraction with trioctyl amine, Bioprocess Eng. 19 (1998) 317.

[38] C.S. López-Garzón, A.J.J. Straathof, Recovery of carboxylic acids produced by fermentation, Biotechnol. Adv. 32 (2014) 873–904.

[39] A. Krzyzaniak, M. Leeman, F. Vossebeld, T.J. Visser, B. Schuur, A.B. De Haan, Novel extractants for the recovery of fermentation derived lactic acid, Sep. Purif. Technol. 111 (2013) 82–89.

[40] F.S. Oliveira, J.M.M. Araújo, R. Ferreira, L.P.N. Rebelo, I.M. Marrucho, Extraction of l-lactic, l-malic, and succinic acids using phosphonium-based ionic liquids, Sep. Purif. Technol. 85 (2012) 137–146.

[41] J. Marták, Š. Schlosser, Extraction of lactic acid by phosphonium ionic liquids, Sep. Purif. Technol. 57 (2007) 483–494.

[42] A. Krzyzaniak, B. Schuur, A.B. De Haan, Equilibrium studies on lactic acid extraction with N,N-didodecylpyridin-4-amine (DDAP) extractant, Chem. Eng. Sci. 109 (2014) 236–243.

[43] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using Lactobacillus casei ATCC 393, Dyna. 85 (2018) 360–366.

[44] A.D. Pérez, V.M. Gómez, S. Rodríguez-Barona, J. Fontalvo, Liquid-liquid Equilibrium and

Molecular Toxicity of Active and Inert diluents of the Organic Mixture Tri-iso-octylamine/Dodecanol/Dodecane as Potential Membrane Phase for Lactic Acid Removal, J. Chem. Eng. Data. submited (2019).

[45] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria of Lactic Acid/Water Solutions in Tri-iso-octylamine/Dodecane/1-Dodecanol at 306.1, 310.1, and 316.1 K. Experimental Data and Prediction, J. Chem. Eng. Data. 64 (2019) 603-610.

[46] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[47] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[48] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702.

[49] G. Chronopoulos, A. Bekatorou, E. Bezirtzoglou, A. Kaliafas, A.A. Koutinas, R. Marchant, I.M. Banat, Lactic acid fermentation by Lactobacillus casei in free cell form and immobilised on gluten pellets, Biotechnol. Lett. 24 (2002) 1233–1236.

[50] O. Sejong, R. Sungsue, S. Jaehun, K. Sangkyo, B. Yungjin, Optimizing conditions for the growth of Lactobacillus casei YIT 9018 in Tryptone-Yeast Extarct- Glucose Medium by using response surface methodology, Appl. Environ. Microbiol. 61 (1995) 3809–3814.

[51] P.S. Panesar, J.F. Kennedy, C.J. Knill, M. Kosseva, Production of L(+) Lactic Acid using Lactobacillus casei from Whey, Brazilian Arch. Biol. Technol. 53 (2010) 219–226.

[52] A.D. Pérez, B. Van der Bruggen, J. Fontalvo, Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation, Chem. Eng. Process. - Process Intensif. 134 (2018) 20–27.

[53] G.F. Teletzke, H.T. Davis, L.E. Scriven, Wetting hydrodynamics, Rev. Phys. Appliquée. 23 (1988) 989–1007.

[54] H. V. Nickens, D.W. Yannitell, The effects of surface tension and viscosity on the rise velocity of a large gas bubble in a closed, vertical liquid-filled tube, Int. J. Multiph. Flow. 13

(1987) 57–69.

[55] J.D. Tice, A.D. Lyon, R.F. Ismagilov, Effects of viscosity on droplet formation and mixing in microfluidic channels, Anal. Chim. Acta. 507 (2004) 73–77.

[56] M.N. Kashid, D.W. Agar, S. Turek, CFD modelling of mass transfer with and without chemical reaction in the liquid-liquid slug flow microreactor, Chem. Eng. Sci. 62 (2007) 5102–5109.

[57] N. Aoki, S. Tanigawa, K. Mae, A new index for precise design and advanced operation of mass transfer in slug flow, Chem. Eng. J. 167 (2011) 651–656.

[58] C. Butler, E. Cid, A.M. Billet, Modelling of mass transfer in Taylor flow: Investigation with the PLIF-I technique, Chem. Eng. Res. Des. 115 (2016) 292–302.

[59] M. Mendorf, D.W. Agar, Scale-up of Capillary Extraction Equipment, Chemie Ing. Tech. 83 (2011) 1120–1124.

[60] J. Fontalvo, M. a. G. Vorstman, J.G. Wijers, J.T.F. Keurentjes, Heat supply and reduction of polarization effects in pervaporation by two-phase feed, J. Memb. Sci. 279 (2006) 156–164.

[61] R. Juang, S. Lee, R. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239.

4.2 Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation[6]

Abstract

The use of a liquid membrane in Taylor flow regime is a recent technology, which extends and generalizes the definition of a membrane. It has been developed and tested for lactic acid removal. A challenge in understanding the technology is that the values of the overall volumetric mass transfer coefficients are not known, and it is unclear how they are related with the operational conditions. In this work, the overall volumetric mass transfer for the liquid membrane in Taylor flow was calculated from experimental results and three empirical models, two of which are from literature and one was developed in this work based on dimensional analysis. From combination of experimental results and the developed models the main variables (operational conditions) of the liquid membrane in Taylor flow that have a strong influence on the overall volumetric mass transfer coefficients (in the donor and in the membrane phase) were defined. The relative velocity results as the variable that has the largest influence on the performance of this new liquid membrane technology.

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4.2.1 Introduction

Liquid membrane in Taylor flow regime (LMTF) is a recently developed separation technology [1,2] that uses the Taylor flow regime as a contact media among the phases of a liquid membrane taking advantage of the enhanced mass transfer of this type of two-phase flow (extended to a three phases system). In the LMTF the solute is transported from the donor drops to the receiving drops through the membrane, which is the continuous phase [1,2].

Nowadays several studies of mass transfer in liquid-liquid systems have been developed. One of the most applied two-phase flow regimes is Taylor flow (also denoted as slug flow, plug flow or segmented flow). Taylor flow enhances mass and heat transfer even in laminar conditions [3,4] due to the presence of a continuous phase film that separates the droplets from the channel wall [5], internal recirculation within the slug and the droplets that are induced by wall shear [6–8], which enhances diffusive penetration [9] and renews the interfaces [5], in addition to the high specific interfacial areas in this kind of two-phase flow [7,8]. Understanding the enhanced potential for mass transfer of Taylor flow, several studies have focused on the development of new microdevices that enhance the mass transfer, such as micromixers [10–12], twisted channels [13], caterpillar channels [14], microstructured reactors [15–17], while others were focused on the development and testing of devices for the separation of the involved phases in these systems [4,5,12,17–20].

Most of these studies considered the overall mass transfer coefficient (OVMTC) in liquid-liquid flow regimes in order to quantify the mass transfer. The overall mass transfer coefficient is a parameter or index to compare how much the mass transfer has been increased using a given two-phase flow regime, among the types of microdevices, and depending on the kind of operation used in these systems. OVMTC values have been calculated from experimental results through the mass balance from the drop or the continuous phase [15]. Extraction of phenol in dodecane to water phase was studied through OVMTC values calculated from the temporal change of the concentration of extracted phenol in the water phase using slug flow, testing the influence of expansions and contractions of the microchannel on mass transfer [21]. The same system was evaluated in circular and semicircular microchannels through the OVMTC achieved from the mass balance for the change of concentration of phenol [22]. The OVMTC was calculated from the mass flux through the interface droplet-continuous phases in square microchannels for the system water/acetone/toluene with acetone as solute in slug flow [4]. The same system was evaluated in different types of microchannels and at several two-phase flows including slug flow [14]. The microfluidic extraction of the systems water/phenol/n-hexane, water/acetone/toluene, water/succinic acid/n-octanol and

water/succinic acid/n-butanol was tested using the OVMTC [23]. Several operational conditions were tested for the systems water/acetone/toluene and water/acetic acid/kerosene in microcapillary contactor using Taylor flow [19]. Extraction of acetone in the system n-butyl acetate/acetone/water was tested using slug flow in different microstructured devices [17]. The removal of some organic acids was tested using OVMTC values in systems such as water/succinic acid/n-butanol from CFD simulations using slug flow [24], aqueous sodium hydroxide/acetic acid/n-hexane for non-circular microchannels with three junctions [11], aqueous sodium hydroxide/tricloroacetic acid/n-hexane using slug flow in circular microchannels at several surfactant concentrations (sodium dodecyl sulfate) [25], aqueous lactic acid/tri-octylamine/n-octanol in slug flow within capillary microreactors (using the mean concentration difference as driving force for the OVMTC) [20], water/succinic acid/n-butanol at different types of microchannels (using the mean concentration difference for the OVMTC calculations) [12], water/toluene/tricoloracetic acid and water/n-hexane/tricoloracetic acid in rectangular microchannels at slug and parallel flows [26], water/succinic acid/1-butanol at several two-phase flow regimes including slug flow using corning microreactors [27], and the system kerosene/acetic acid/water through slug flow in a microstructured reactor [16]. Various microreactors and micromixers were tested using the system n-butanol/toluene/4-nitrophenyl acetate for several liquid-liquid flow regimes [28]. Slug and drop flow mass transfer were tested for the systems aqueous sodium hydroxide/n-butanol/4-nitrophenyl acetate and aqueous sodium hydroxide/toluene/4-nitrophenyl acetate in different microreactors [29]. Extraction of copper ions from nitric acid using a tributylphosphate and ionic liquids mixture was evaluated at different channel size [5]. Extraction of copper was also tested with di-(2-ethylhexyl)phosphoric acid/kerosene mixture in twisted micromixers [13]. The extraction of NBF ester from n-butyl formate to an aqueous solution of sodium hydroxide was carried out using a microreactor and it was tested using OVMTC with the logarithmic-mean of concentrations as driving force of transport of NBF [30].

Additionally, some studies have used or developed empirical correlations in order to predict the overall volumetric mass transfer coefficient. M. Kashid *et al*. [16] proposed a correlation for mass transfer in microstructured reactors using dimensional analyses through the Buckingham Pi method and applied this method to the system kerosene/acetic acid/water where the predicted values were in a good agreement with the experimental values (with R^2 of 0.95). The aforementioned model was used in other work [15] in order to predict the values of the OVMTC in liquid-liquid systems using microstructured reactors. Di Miceli Raimondi *et al.* [31] developed numerical simulations of mass transfer in slug flow; from these results they calculated the OVMTC and compared it with the values

achieved by the models of Skelland and Wellek (for liquid-liquid systems) [32], Berčič and Pintar (for gas-liquid systems) [33] and van Baten and Krishna (for gas-liquid systems) [34] finding less scattering and a better fit through the Skelland and Wellek model. Tsaoulidis *et al*. [5] compared the experimental values of OVMTC with existing models for gas-liquid and liquid-liquid two-phase flows of the literature, such as Vandu *et al*. (for gas-liquid systems) [35], Van Baten and Krishna [34], and Kashid *et al*. [16], achieving the best fit with the latter model. The experimental OVMTC values of an aqueous sodium hydroxide/acetic acid/n-hexane system for non-circular microchannels [11] were compared with predicted values of reported models in the literature [16,31,32], achieving a good fit, especially the model of Kashid *et al*. [16]. Di Miceli Raimondi *et al*. [4] predicted the OVMTC of acetone in the system water/acetone/toluene using several empirical correlations [32–34] including one of their previous work [31], which gave the best fit. In another study, the influence of sodium dodecyl sulfate on the mass transfer of tricloroacetic acid from n-hexane to aqueous sodium hydroxide in a microchannel using Taylor flow was investigated through OVMTC calculations [25]. Furthermore, several empirical correlations [16,33,35,36] were used and the correlation with the best fit was the correlation developed by Kashid *et al*. [16].

Prediction of the OVMTC through empirical correlations allowed for an understanding of the influence of the operational conditions as well as the physical properties of the fluid on the mass transfer in microdevices working under liquid-liquid flow regimes. These models, for liquid-liquid systems, generally include dimensionless numbers, such as Reynolds and Capillary numbers, involving both operational conditions and physical properties. However, its level of influence on the prediction of the OVMTC has varied from work to work, where the type of microdevice used has shown a significate influence on the mass transfer coefficients. On the other hand, these models have allowed the prediction of the OVMTC with a good precision, and can be used for developing simulations of these technologies.

In this work, the overall volumetric mass transfer coefficients of a LMTF were studied at several operational conditions in order to provide an understanding of this membrane technology. Experimental values of the OVMTC for donor and membrane phases were calculated through the flux equation involved for mass transport on slug flow systems. The OVMTC was predicted using two models (as simple methods to quantify the mass transport) that showed a good accuracy in other. One model was developed for the LMTF taking specific parameters of this kind of contact in liquid membranes into account. Predicted values and experimental values were compared.

4.2.2 Theory

Liquid Membrane in Taylor flow regime

In a liquid membrane in Taylor flow (LMTF), the membrane phase is used as a continuous phase (slugs), and the donor and receiving phases are dispersed aqueous phases (droplets), as shown in Fig. 1 [1,2]. All these phases flow within a channel or tube. Solute (*S*) is transferred from the donor phase to the membrane phase and, from here, to the receiving phase. The dispersed phases are separated at the outlet of the tube and the membrane phase is recirculated into the tube.

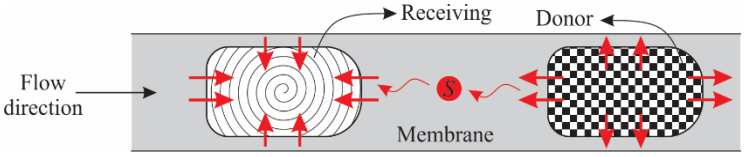


Figure 1. Scheme for the mass transport process through two adjacent droplets (donor and receiving) in a liquid membrane in a Taylor flow system.

Facilitated transport is a mechanism of transport commonly used in liquid membranes. An active agent within the membrane phase reacts with the solute in the interphase donor-membrane producing a complex that is transported to the membrane-receiving interphase. The above transport process occurs faster than the simple diffusion process [10]. Additionally, in spite of the fact that the solute is transported as a complex in facilitated transport, the solute also can diffuse into the membrane phase. Therefore, the solute can be in the membrane phase as a complex and as a single solute. This mechanism of transport takes place in the experiments of the LMTF in this study.

Calculation of the overall volumetric mass transfer coefficients

The overall volumetric mass transfer coefficient can be calculated from the experimental results through a mass balance for the donor phase and for the membrane phase using the flux equation [5,13]. The mass balance for the donor phase for the transport of solute from the bulk of donor phase to donor-receiving interphase is as follows:

$$\frac{dC_D}{dt} = -k_{L,D} a_D \left(C_D - C_D^{eq}\right) \tag{1}$$

In Eq. (1), C_D represents the molar concentration of solute in the donor phase, and C_D^{eq} the equilibrium concentration of the solute in the aqueous phase [13,25] corresponding to an aqueous phase (with a concentration of solute of C_D) in contact with the organic phase (membrane phase).

Integrating Eq. (1) and taking into account the distribution coefficient (*K*) of the liquid-liquid equilibrium (given in Eq. (2)), the overall volumetric mass transfer can be calculated as shown in Eq. (3).

$$K = C_M^{eq} / C_D^{eq} \tag{2}$$

$$\ln\left[\frac{C_D(t_0) - C_M^{eq}/K}{C_D(\tau_D) - C_M^{eq}/K}\right] = k_{L,D} a_D \cdot \tau_D \tag{3}$$

The symbol t_0 represents time at the injection point of the donor phase (0 s) and τ represents the average residence time.

The overall volumetric mass transfer coefficient through the membrane phase, from the bulk of the membrane phase to the membrane-receiving interphase was calculated from a mass balance with the flux equation in steady state, where the flux from donor to membrane phase is the same flux than the flux from the membrane to receiving phase (J_{ss}):

$$J_{ss} = k_{L,M} a_R \left(C_{M,T} - C_R^{eq}\right) \cdot V_M \tag{4}$$

Where $V_{,M}$ is the volume in the membrane phase within the channel, $C_{M,T}$ is the total solute concentration in the membrane phase (free solute and in the complex form) in steady state, calculated from a mass balance of solute between donor and membrane phases (from $t = 0$ till the steady state is reached for every single experiment). The solute concentration in the receiving phase is zero ($C_R^{eq} = 0$) because in the receiving phase there is an active agent that reacts instantaneously with the solute of the membrane phase. Therefore, the final equation for the overall volumetric mass transfer coefficient for the membrane phase is:

$$k_{L,M} a_R = \frac{J_{ss}}{C_{M,T} \cdot V_M} \tag{5}$$

The used parameters for Eqs. (1)-(5) are defined with their respective units in the nomenclature table.

Models for the overall mass transfer coefficient prediction

The model proposed by Di Miceli Raimondi *et al*. [4,31] and used by Sattari-Najafabadi *et al*. [11] for mass transfer in extraction systems in Taylor flow was adapted in order to be applied for

prediction of the overall volumetric mass transfer coefficients of a liquid membrane in Taylor flow regime. Eqs. (6)-(7) show the abovementioned model for prediction of the overall volumetric mass transfer coefficient, for both the donor and membrane phase.

$$k_{L,D}a_D = \delta_1 \cdot \left(\frac{a_D}{d_{dD}}\right) \cdot \left(\frac{V_D}{V_{UCD}}\right)^{\delta_2} \cdot \left(U_{T,D} \cdot d\right)^{\delta_3} \cdot \left(\frac{U_{T,D}}{\gamma}\right)^{\delta_4} \left(\frac{d}{d_{dD}}\right)^{\delta_5} \tag{6}$$

$$k_{L,M}a_R = \delta_1^{'} \cdot \left(\frac{a_R}{d_{dR}}\right) \cdot \left(\frac{V_R}{V_{UCR}}\right)^{\delta_2^{'}} \cdot \left(U_{T,R} \cdot d\right)^{\delta_3^{'}} \cdot \left(\frac{U_{T,R}}{\gamma}\right)^{\delta_4^{'}} \left(\frac{d}{d_{dR}}\right)^{\delta_5^{'}} \tag{7}$$

A second model was adapted from the model used by Kashid *et al*. [15,16] in liquid-liquid extraction process using microreactors. This adapted model is shown in Eqs. (8)-(9) for the overall volumetric mass transfer coefficient of the donor and membrane phases.

$$k_{L,D}a_D \cdot \tau_D = \lambda_1 \cdot \left(Ca_{mix,D}\right)^{\lambda_2} \cdot \left(\mathrm{Re}_{mix,D}\right)^{\lambda_3} \cdot \left(\frac{d}{L}\right)^{\lambda_4} \tag{8}$$

$$k_{L,M}a_R \cdot \tau_R = \lambda_1^{'} \cdot \left(Ca_{mix,R}\right)^{\lambda_2^{'}} \cdot \left(\mathrm{Re}_{mix,R}\right)^{\lambda_3^{'}} \cdot \left(\frac{d}{L}\right)^{\lambda_4^{'}} \tag{9}$$

where L is the channel length and λ and $\lambda^{'}$ are fitted parameters of the model. The dimensionless numbers, Capillary number (*Ca*) and, Reynolds number (Re) were calculated for the respective phase (donor or receiving) at weighted physical properties according to the flow rates [15,16].

$$Ca_{mix,D} = \frac{U_{T,D} \cdot \mu_{mix,D}}{\gamma}; Ca_{mix,R} = \frac{U_{T,R} \cdot \mu_{mix,R}}{\gamma} \tag{10}$$

$$\mathrm{Re}_{mix,D} = \frac{\rho_{mix,D} \cdot U_{T,D} \cdot d}{\mu_{mix,D}}; \mathrm{Re}_{mix,R} = \frac{\rho_{mix,R} \cdot U_{T,R} \cdot d}{\mu_{mix,R}} \tag{11}$$

$$\begin{aligned} \mu_{mix,D} &= \mu_M\left(\frac{Q_M}{Q_M + Q_D}\right) + \mu_D\left(\frac{Q_D}{Q_M + Q_D}\right); \\ \mu_{mix,R} &= \mu_M\left(\frac{Q_M}{Q_M + Q_R}\right) + \mu_D\left(\frac{Q_R}{Q_M + Q_R}\right) \end{aligned} \tag{12}$$

$$\begin{aligned} \rho_{mix,D} &= \rho_M\left(\frac{Q_M}{Q_M + Q_D}\right) + \rho_D\left(\frac{Q_D}{Q_M + Q_D}\right); \\ \rho_{mix,R} &= \rho_M\left(\frac{Q_M}{Q_M + Q_R}\right) + \rho_D\left(\frac{Q_R}{Q_M + Q_R}\right) \end{aligned} \tag{13}$$

where μ, ρ and Q are the viscosity, density and flow rate, respectively, of the donor (D), receiving (R) or membrane phase (M).

Additionally, a third model was developed in this work, knowing that the overall volumetric mass transfer coefficient is function of the following variables: channel length, slug length, total velocity of the dispersed (donor or receiving) and membrane phases, injection time and channel diameter. In addition, it is function of physical properties: viscosity, density and interfacial tension (Eqs. (14) and (15)).

$$k_{L,D}a_D = f\left(L, L_{slug}, U_{T,D}, U_{T,M}, t_{inj}, d, \mu_{mix,D}, \rho_{mix,D}, \gamma\right) \tag{14}$$

$$k_{L,M}a_R = f\left(L, L_{slug}, U_{T,R}, U_{T,M}, t_{inj}, d, \mu_{mix,R}, \rho_{mix,R}, \gamma\right) \tag{15}$$

Using dimensional analysis through the Rayleigh method [37] the following equations for the overall volumetric mass transfer coefficients were derived:

$$k_{L,D}a_D = \alpha_1 \cdot \left(\frac{U_{T,D}}{d}\right) \cdot \left(\mathrm{Re}_{mix,D}\right)^{\alpha_2} \cdot \left(Ca_{mix,D}\right)^{-\alpha_3} \cdot \left(1 - W_D\right)^{\alpha_4} \cdot \left(\frac{U_{T,D} \cdot t_{inj}}{d}\right)^{\alpha_5} \cdot \left(\frac{L}{d}\right)^{\alpha_6} \cdot \left(\frac{L_{slug}}{d}\right)^{\alpha_7} \tag{16}$$

$$k_{L,M}a_R = \alpha_1^{'} \cdot \left(\frac{U_{T,R}}{d}\right) \cdot \left(\mathrm{Re}_{mix,R}\right)^{\alpha_2^{'}} \cdot \left(Ca_{mix,R}\right)^{-\alpha_3^{'}} \cdot \left(1 - W_R\right)^{\alpha_4^{'}} \cdot \left(\frac{U_{T,R} \cdot t_{inj}}{d}\right)^{\alpha_5^{'}} \cdot \left(\frac{L}{d}\right)^{\alpha_6^{'}} \cdot \left(\frac{L_{slug}}{d}\right)^{\alpha_7^{'}} \tag{17}$$

where t_{inj}, is the injection time of the respective dispersed phase, α and $\alpha^{'}$ are the fitted parameters. This model includes the dimensionless number relative velocity (W) that was used by Taylor [38] to propose an empirical relationship between this dimensionless number and the capillary number in which the flow patterns of slug flow could be predicted.

The dimensionless relative velocity is calculated as follows:

$$W_D = \frac{U_{T,D} - U_{T,M}}{U_{T,D}}; W_R = \frac{U_{T,R} - U_{T,M}}{U_{T,R}} \tag{18}$$

4.2.3 Materials and methods

Materials

Tri-iso-octylamine (TiOA- assay 95%), n-dodecane (assay 99%), 1-dodecanol (assay 98%), and sodium carbonate (assay 99.5%) were supplied by Merck Millipore. L(+)-lactic acid was supplied by Panreac Química S.A.U. (assay 88.0-92.0%). The purity of lactic acid was assessed by titration with sodium hydroxide of Carlo Erba (assay ≥ 97.0%) using a Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). A stock solution of lactic acid (150 g/L) were heated at 90 °C under total reflux between 8-10 hours for dimer hydrolysis [39,40] and subsequently, the lactic acid concentration was measured by titration. Type I water was used for all aqueous solutions (Barnstead™ Nanopure™).

Experiments of the liquid membrane in Taylor flow regime

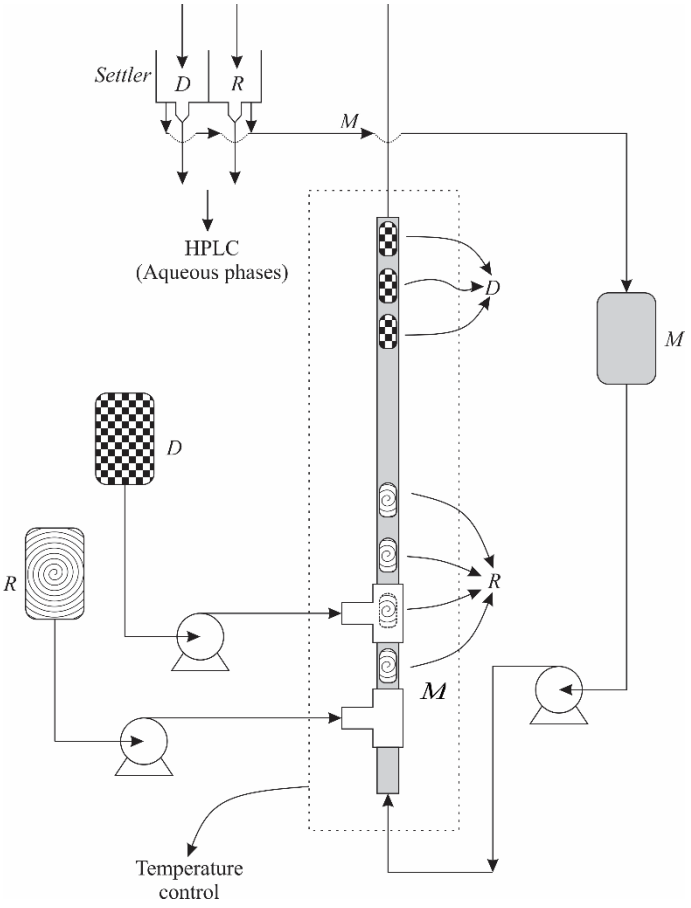


Figure 2. Experimental setup used in the LA removal with a LMTF.

The LMTF was tested for lactic acid (LA) removal. The membrane phase was a mixture of Tri-iso-octylamine, dodecanol and dodecane at 10, 40 and 50 vol%, respectively. The donor phase was an aqueous solution of LA at 10 g/L prepared from the stock solution. The receiving phase was an aqueous solution of sodium carbonate at 2.5 g/L.

In this system, TiOA is a carrier that reacts with the LA from the donor phase in order to produce an LA-TiOA complex. This LA-TiOA complex is transported to the membrane-receiving interphase where an instantaneous acid-base reaction is carried out between the sodium carbonate and the LA of the LA-TiOA complex. Additionally, a small amount of free LA is solubilized in the membrane phase that also reacts with the sodium carbonate in the membrane-receiving interface as well.

Table 1. Experimental conditions tested for lactic acid removal with a LMTF system.

Membrane flow rate, Q_M (cm^3/s)	**Donor flow rate, Q_D (cm^3/s)**	**Receiving flow rate, Q_R (cm^3/s)**	**Injection time, t_{inj} (s)**	**Delay time, t_{del} (s)**
0.0750	0.0333	0.0193	6	6
0.0750	0.0333	0.0193	12	12
0.0750	0.0333	0.0193	16	16
0.1083	0.0333	0.0173	6	6
0.1083	0.0333	0.0173	12	12
0.1083	0.0333	0.0173	16	16
0.1417	0.0333	0.0122	6	6
0.1417	0.0333	0.0122	12	12
0.1417	0.0333	0.0122	16	16
0.1650	0.0333	0.0118	6	6
0.1650	0.0333	0.0118	12	12

In the experiments a transparent polyurethane circular channel (tubing) of 2.5 mm of inner diameter and 348.8 cm of length, coiled in a vertical length of 45 cm was used. For the flow of the membrane phase a HPLC pump (Waters 501) was used. The injection of donor phase was carried out through a syringe pump (Cole-Parmer® Touch Screen Infuse/Withdraw) and for the receiving phase was used a gear pump (Ismatec, Reglo-z) with a pump-head (Ismatec Z-186, at a fixed flow rate of 5%) coupled to a solenoid valve (STNC® - DC 24V). The injection time of the donor phase was programmed in the syringe pump. The receiving phase injection was controlled by the open-close times of the solenoid valve Through a Arduino Mega. For separation of the donor and receiving droplets, a settler with two separate compartments (Figure 2), one for each aqueous phase, was used. No entrainment is possible between the two compartments. Due to the aqueous phases are already

separated in droplets from the organic phase, the residence time for separation in each compartment is short and lower than 5 s. Donor and receiving phases are injected at specific times and between injections, there is a delay time (6 – 16 s) where no aqueous phase injection takes place. For a given injection time, for a train of droplets, of donor or receiving phase, and the residence time (85 – 160 s), it was possible to know the time where a given phase (donor or receiving) arrived at the top of the channel. Consequently, the train of droplets at the top of the channel is fed to the corresponding settler using a flexible tubing. The time required for the flexible tubing to shift to the corresponding compartment is lower (1 s) than the delay time. The organic phase split from each compartment was recycled to the inlet of the channel.

Several flow rates of the membrane phase, and of the dispersed phases (donor and receiving) were tested at several injection and delay times. The delay time is the elapsed time from the end of the injection of the donor phase until starts the injection of the receiving phase. The tested experimental conditions are shown in Table 1. In each injection of the respective dispersed phase, a train of droplets is formed, as shown in Fig. 2.

During the constant flow of the membrane phase within the channel, each injection cycle follows the following four steps: First, the donor phase is injected during t_{inj}. Then the injection of the donor phase is stopped and the delay time (t_{del}) elapses. Afterwards, the receiving phase start to inject during t_{inj}, and in the fourth step, the receiving phase injection is stopped and the delay time elapses again. The aforementioned cycle is repeated along the experiment time (between 30 and 50 min). In each injection of the donor or receiving phase, a train of droplets of the respective phase is formed (Fig. 3). The flow rate of the donor phase was kept at the same value, thus the variation of the injected volume of the donor phase is only due to the injection time. For the receiving phase, the flow rate was kept 5%, however, the injected volume of this phase depends on the injection time and the flow rate of the membrane phase. The same value of the injection time was used for the delay time for each experiment.

Two kinds of slug are formed during the process as shown in Fig. 3 and each one has its respective length. There is a slug length between the back cap of the last droplet of a train of droplets of the donor phase and the front cap of the first droplet of the subsequent train of droplets of the receiving phase. Another slug is located into the train of droplets of the same phase, and its length is given between the back cap of a droplet and the front cap of the subsequent droplet.

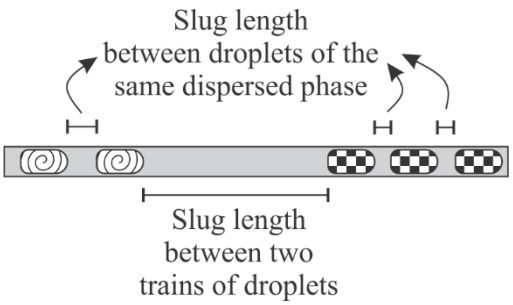


Figure 3. Scheme of the slug lengths that are formed in the LMTF system.

For each experiment, the LA concentration was measured from the samples of the donor and receiving phases that were collected in the corresponding container of the settler. HPLC method was used for LA concentration measurements [2]. These samples were taken at long the time of the experiment until the concentration of LA in the aqueous phases was constant (where the steady state is achieved), at that time the experiment was stopped. Every experiment was carried out at 37 °C.

The system reaches a steady state operation. The steady state is reached when the amount of LA removed from the donor phase equals the amount of LA that is captured by the receiving phase. So, in steady state, the LA concentration in the organic phase is relatively low but far from equilibrium. The amount of LA in the organic phase fed to the channel, which is reached at steady state, depends on the specific operating conditions. LA concentration was measured by HPLC from samples of the aqueous phases in each compartment as a function of time till the concentrations were constant and the steady state was reached. HPLC chromatograms for samples coming from the receiving phase present a small pick due to the salt. This small pick is not shown for samples coming from the donor phase

Physical properties calculation

Density and the viscosity of the fed donor and receiving phases were measured experimentally at 37 °C using a pycnometer of 5 mL (Vilab) and an Ubbelohde viscometer (Cannon), respectively.

Physical properties at 37 °C of tri-iso-octylamine [41], dodecane [42] and dodecanol [43] were taken from literature. The membrane phase density and viscosity were calculated as a function of the volumetric fraction of each substance:

$$\rho_M = 0.1 \cdot \rho_{TiOA} + 0.5 \cdot \rho_{Dod} + 0.4 \cdot \rho_{DOH} \quad (19)$$

$$\mu_M = 0.1 \cdot \mu_{TiOA} + 0.5 \cdot \rho_{Dod} + 0.4 \cdot \mu_{DOH} \quad (20)$$

The interfacial tension was calculated as average from the literature values of dodecane-water [44] and dodecanol-water [45] systems at 37 °C, because dodecanol and dodecane are the substance of the highest concentration in the membrane phase and lactic acid is a low concentration in the aqueous phase.

Fit of the overall volumetric mass transfer coefficient from the models

Fitting of the aforementioned models was achieved using Matlab® (with *globalsearch* function) through the average absolute relative deviation (*AARD*) showed in Eq. (21).

$$AARD = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{\left|\left(k_L a\right)_{\exp,i} - \left(k_L a\right)_{calc,i}\right|}{\left(k_L a\right)_{\exp,i}}\right) \tag{21}$$

where N is the number of data (experiments) and i is the i-th experiment. A Matlab® script was developed using the *globalsearch* function to minimize the equation (21). The experimental values of the OVMTC were achieved from equations (3) and (5), for solute transport from the bulk of the donor phase to the donor-membrane interphase and for solute transport from the bulk of membrane phase to the membrane-receiving interphase, respectively. The calculated values were achieved from equations (6), (8) and (16) for the solute transport from the bulk of the donor phase to the donor-membrane interphase and from equations (7), (9) and (17) for solute transport from the bulk of membrane phase to the membrane-receiving interphase depending of the model used (one of the two taken from the literature or the developed model in this work).

4.2.4 Results and discussion

Calculation of experimental overall volumetric mass transfer coefficients

The overall volumetric mass transfer coefficients were calculated from Eqs. (3) and (5), in the donor phase and in the membrane phase, respectively. The velocity of each phase was calculated from the tested volumetric flow rates. In Fig. 4 the influence of the droplet velocity on the overall volumetric mass transfer coefficient (OVMTC) is shown. For both overall volumetric mass transfer coefficients in Fig. 4, the higher the droplet velocity (donor or receiving), the higher the OVMTC. A high velocity of the droplet could decrease the mass transfer resistance that is due to the viscosity (shear stress) of the fluids. Furthermore, the higher the droplet velocity, the higher the interfacial velocity of the droplets, which could provide a higher mixing velocity (vortices of higher velocity) that consequently increases the mass transfer. On the other hand, the liquid surrounding the donor droplet

is renewed faster with this increase in the droplet velocity, which involves a higher gradient of lactic acid concentration between the aqueous and membrane phase [46].

In Taylor flow regime, generally, a change in the droplet velocity involves changes in the shape of the droplet. From the Bretherton law's is known that the higher the droplet velocity, the higher the film thickness around the droplet [9,15,30] and in consequence, the droplet length rises as well and the droplet diameter decreases. The aforementioned involves a change in the interfacial area of the droplet, but not necessarily it increases with the change of the drop length and decreases of the droplet diameter. However, the interfacial area of the droplet increases as the droplet velocity rises [5], because of the higher the droplet velocity, the lower the slug length (due to a faster penetration velocity of the dispersed phase into the continuous phase) between the droplets of the same phase which means an increase of the amount of droplets in the same channel length.

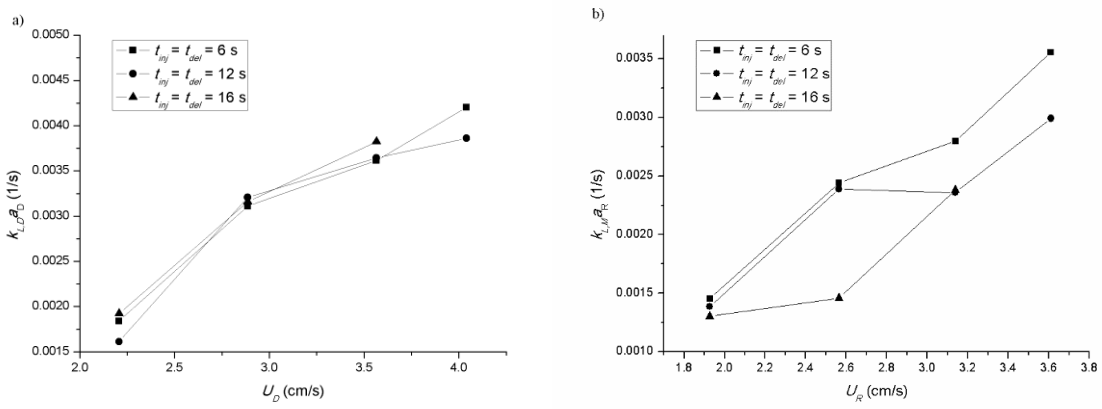


Figure 4. Overall volumetric mass transfer coefficients as function of droplet velocity at three injection-delay times (6, 12 and 16 s).

According to Fig. 4a, the effect of the injection and delay time could be negligible for all donor droplet velocities, and for receiving droplet velocities between 1.9 and 2.6 cm/s. For all droplet velocities beyond 3.1 cm/s for the receiving phase, there is a higher influence of the injection and delay time than below this value. At the highest experimentally velocity of the droplet, for both the donor and receiving phase, the lower the injection time, the higher the OVMTC. However, for the highest delay time (16 s), the OVMTC for membrane phase, is the lowest compared with the delay times of 6 s and 12 s (which have similar values in between).

Changes in slug length (Fig. 5) are due to both the delay time and the droplet velocity. The higher the droplet velocity and the higher the delay time, the higher the slug length. In order to understand which of both variables affect the OVMTC the most, the effect of the slug length at fixed values of

the droplet is shown in Fig. 5. From these figures, two observations can be made. The higher the droplet velocity, the higher the OVMTC. Secondly, at a fixed value of the velocity, the change of the OVMTC with the slug length is almost negligible. Thus, the droplet velocity is the variable that has the highest influence on the OVMTC, rather than the delay time.

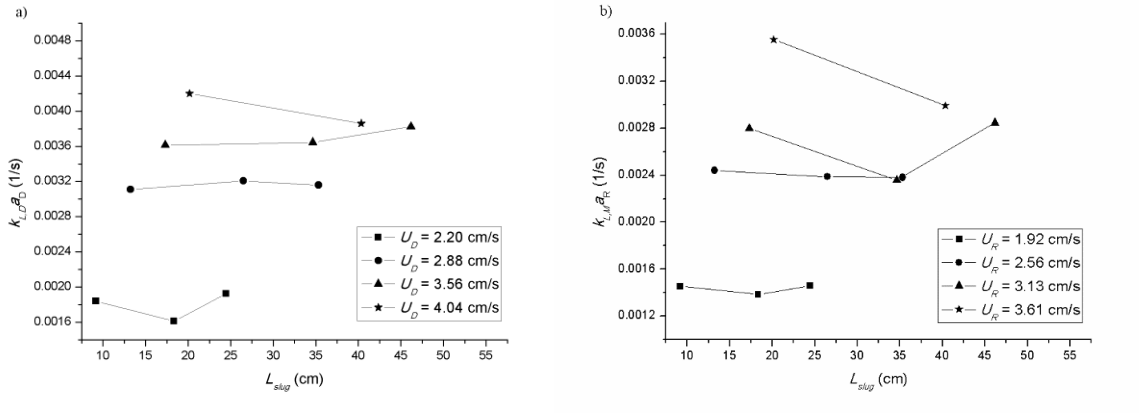


Figure 5. Overall volumetric mass transfer coefficients as function of slug length at its respective droplets velocity.

For all droplet velocities of the donor phase and at low droplets velocities of the receiving phase (lower than 2.56 cm/s) there was no effect of the injection and delay time on the OVMTC, while at high droplet velocities of the receiving phase (beyond 2.56 cm/s) there is a slight effect. This may be because at low droplet velocities, the residence time of the droplets is high enough to achieve mass transfer. In this way, the residence time of the droplets is affected by the droplet velocity and for the channel length. In the experiments, the length channel was constant (348.8 cm), therefore, the higher the droplet velocity, the lower the residence time of the droplets. Low residence times do not allow for the entire mass transfer process.

Models for overall volumetric mass transfer coefficients prediction

The three proposed models were fitted using the experimental results of the overall volumetric mass transfer coefficients using Eqs. (6)-(7) for adapted model 1, Eqs. (8)-(9) for adapted model 2 and Eqs. (16)-(17) for the model proposed in this work. The calculated density and viscosity of the membrane phase was 779.756 kg/m^3 and 0.0054 Pa·s, respectively at 37 °C. The interfacial tension at 37 °C was 30.44 mN/m. The density and viscosity for the donor phase was 1001.3 kg/m^3 and 0.0012 Pa·s, respectively, and for the receiving phase 1023.9 kg/m^3 and 0.7669 Pa·s, respectively.

The comparison of the three models with the experimental results is shown in Fig. 6. Fitted parameters and the values of the R^2 of each model are shown in Table 2. For the adapted model 2 the same values of the calculated OVMTC can be obtained for different values of the experimental OVMTC. This is because this model is fully dependent on the droplet velocity, through Reynolds and Capillary numbers, and the residence time. Thus, for a fixed value of droplet velocity, the same value of the OVMTC with this model is achieved (circles in Fig. 6). On the other hand, the adapted model 1 is not only a function of the droplet velocity, but also of the injected volume of the dispersed phase, which allows studying variations of the calculated OVMTC at a fixed value of the droplet velocity. For this reason, the R^2 value for the OVMTC of the membrane phase of the adapted model 1 was higher than for the adapted model 2, but in spite of this, the value of R^2 for the OVMTC of the donor phase was similar for both models (slightly higher for the adapted model 2 than for the adapted model 1).

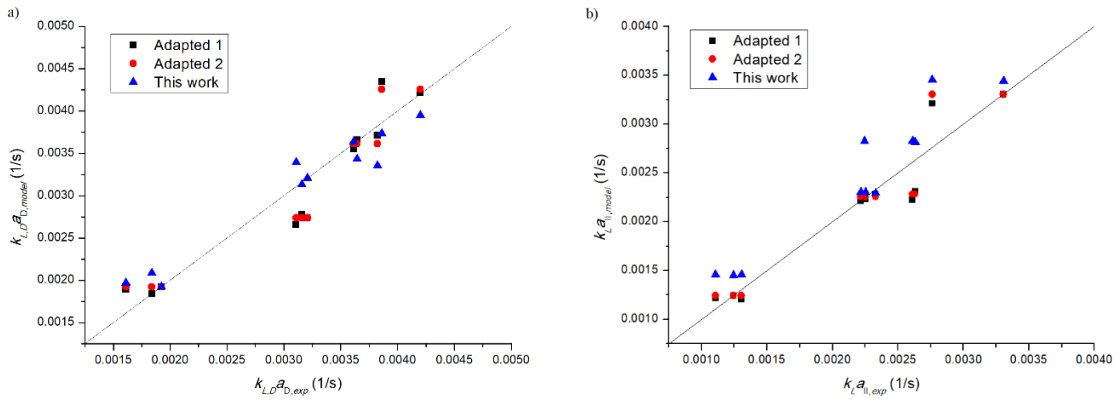


Figure 6. Comparison of the experimental overall volumetric mass transfer coefficients with achieved by models.

The model developed in this study has less scattering of the data and represents the best fit. This may be related to the fact that this model includes variables such as injection time, slug length, and the dimensionless relative velocity, while the other models are a function of less variables. Experimentally, these variables showed a high influence on the OVNTC in the LMTF.

In the model developed in this work, the highest scattering of the data is observed at the highest values of the OVMTC where the experimental flow rate of the membrane phase was 9.9 mL/min (with an injection time of 6 s), while for low and medium values of the OVMTC the dispersion is low where the experimental membrane flow rate was 4.5, 6.5 and 8.5 ml/min.

According to the values of the fitted parameters (the power of the models), the OVMTC (donor and membrane) shown a high dependency on the product of the droplet velocity and the channel diameter for the adapted model 1. Additionally, in the OVMTC for the donor phase there was an appreciable influence of the term of the ratio equivalent diameter to inner diameter of the channel, while for the membrane phase there was an appreciable influence of the ratio of the receiving droplet volume to unit cell receiving phase and the ratio of receiving droplet velocity to interfacial tension. In the adapted model 2, the OVMTC for both donor and membrane phase shows a high dependency on the Capillary number.

Table 2. Fitted parameters of the three models for the OVMTC of donor and membrane phase and its respective values of R^2.

Model	**R^2**	**Fitted parameters**							
		j	1	2	3	4	5	6	7
Adapted 1	0.888	δ_j	0.0776	0.3342	1.7251	-0.0192	-1.1646	-	-
	0.901	δ_j'	0.0018	1.1103	1.0891	2.2484	-0.9758	-	-
Adapted 2	0.896	λ_j	2.1588	0.2018	0.0942	0.1404	-	-	-
	0.885	λ_j'	0.7188	0.0796	0.1453	0.0850	-	-	-
This work	0.924	α_j	0.0003	-1.5002	1.7079	20.4570	-1.1497	-0.2303	1.0673
	0.913	α_j'	0.0014	0.1679	-0.7666	2.0224	0.0309	-0.6909	-0.0390

In the model developed in this work, some variables such as, slug length, injection time and relative velocity, are involved that are not included in the adapted models 1 and 2. These variables showed an appreciable influence on the OVMTC. In the donor and membrane phase, the OVMTC showed the highest dependency on the relative velocity. In the donor phase the highest dependency was found for the reverse of the Reynolds and Capillary numbers, the ratio slug length to inner diameter and the ratio inner diameter to the product donor droplet velocity to injection time. These parameters are function of the donor velocity, physical properties of the fluids (density, viscosity and interfacial tension) and slug length. On the other hand, in the membrane phase, the OVMTC was influenced by the Capillary number, and the ratio inner diameter to channel length.

4.2.5 Conclusions

Overall volumetric mass transfer coefficients (OVMTC) were calculated from experimental results, and three empirical correlations were fitted for the OVMTC prediction. From both experimental OVMTC and correlations, the influence of some variables of the system was observed.

According to the experimental results, the variables that affect the overall volumetric mass transfer coefficient the most were droplet velocities and injection time. The higher the droplet velocity and the lower the injection time, the higher the overall mass transfer coefficient for both the donor and membrane phase.

The OVMTC for the membrane phase is directly influenced by the OVMTC of the donor phase, because the amount of lactic acid available to be transported from the membrane phase to the receiving phase depends on the transport process of lactic acid from the donor to membrane phase.

The OVMTC can be predicted with a good agreement (R^2 of 0.92) for donor phase and membrane phase (R^2 of 0.91). In the proposed model of this work the donor droplets velocity, relative velocity for donor phase, injection time, slug length, density, viscosity, and interfacial tension have a high influence on the OVMTC of the donor phase. The receiving droplet velocity, channel length (that means residence time), relative velocity for receiving phase, the viscosity of the fluids and interfacial tension have a high influence on the OVMTC of the membrane phase.

NOTATION

a	Specific surface area (m^2/m^3)
C	Molar concentration of solute (mol/L)
Ca	Capillary number
d	Inner diameter of the channel (m)
d_d	Droplet equivalent diameter (m)
J_{ss}	Steady state flux of solute through the membrane phase
K	Distribution coefficient
k_La	Overall volumetric mass transfer coefficient (1/s)
L	Channel length (m)
Q	Volumetric flow rate (m^3/s)
Re	Reynolds number
S	Solute
t	Time (s)
U	Droplet velocity (m/s)
V	Volume (m^3)
W	Relative velocity
α	Fitted parameter of the developed model
δ	Fitted parameter of the adapted model 1
γ	Interfacial tension (N/m)
λ	Fitted parameter of the adapted model 2
μ	Viscosity (Pa·s)
τ	Residence time (s)
ρ	Density (kg/m^3)

Subscripts and superscripts

0	Initial
calc	Calculated data
del	Delay
D	Donor phase
eq	In equilibrium
exp	Experimental data
inj	Injection
mix	Mixture property between the disperse and continuous phases
M	Membrane phase
R	Receiving phase
T	Total
UC	Unit cell between the respective dispersed phase and the membrane phase

4.2.6 References

[1] J. Fontalvo, A.D. Pérez, Membrana Líquida y proceso para realizarlo, Colombian pending patent, Rad. 15-131023.

[2] A.D. Pérez, Desarrollo y evaluación de un sistema de membrana líquida en flujo de Taylor para la remoción de ácido láctico, Universidad Nacional de Colombia, 2014.

[3] M.T. Kreutzer, F. Kapteijn, J.A. Moulijn, C.R. Kleijn, J.J. Heiszwolf, Inertial and interfacial effects on pressure drop of Taylor flow in capillaries, AIChE J. 51 (2005) 2428–2440.

[4] N. Di Miceli Raimondi, L. Prat, C. Gourdon, J. Tasselli, Experiments of mass transfer with liquid-liquid slug flow in square microchannels, Chem. Eng. Sci. 105 (2014) 169–178.

[5] D. Tsaoulidis, P. Angeli, Effect of channel size on mass transfer during liquid-liquid plug flow in small scale extractors, Chem. Eng. J. 262 (2015) 785–793.

[6] R.S. Abiev, Bubbles velocity, Taylor circulation rate and mass transfer model for slug flow in milli- and microchannels, Chem. Eng. J. 227 (2013) 66–79.

[7] R. Gupta, D.F. Fletcher, B.S. Haynes, Taylor Flow in Microchannels: A Review of Experimental and Computational Work, J. Comput. Multiph. Flows. 2 (2010) 1–31.

[8] A. Ufer, M. Mendorf, A. Ghaini, D.W. Agar, Liquid-Liquid Slug Flow Capillary Microreactor, Chem. Eng. Technol. 34 (2011) 353–360.

[9] M.N. Kashid, I. Gerlach, S. Goetz, J. Franzke, J.F. Acker, F. Platte, D.W. Agar, S. Turek, Internal circulation within the liquid slugs of a liquid-liquid slug-flow capillary microreactor,

Ind. Eng. Chem. Res. 44 (2005) 5003–5010.

[10] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[11] M. Sattari-Najafabadi, M. Nasr Esfahany, Z. Wu, B. Sundén, Hydrodynamics and mass transfer in liquid-liquid non-circular microchannels: Comparison of two aspect ratios and three junction structures, Chem. Eng. J. 322 (2017) 328–338.

[12] K.K. Singh, A.U. Renjith, K.T. Shenoy, Liquid-liquid extraction in microchannels and conventional stage-wise extractors: A comparative study, Chem. Eng. Process. Process Intensif. 98 (2015) 95–105.

[13] O. Jafari, M. Rahimi, F.H. Kakavandi, Liquid-liquid extraction in twisted micromixers, Chem. Eng. Process. Process Intensif. 101 (2016) 33–40.

[14] M. N. Kashid, A. Renken, L. Kiwi-Minsker, Influence of Flow Regime on Mass Transfer in Different Types of Microchannels, Ind. Eng. Chem. Res. 50 (2011) 6906–6914.

[15] M.N. Kashid, A. Renken, L. Kiwi-Minsker, Gas-liquid and liquid-liquid mass transfer in microstructured reactors, Chem. Eng. Sci. 66 (2011) 3876–3897.

[16] M.N. Kashid, A. Gupta, A. Renken, L. Kiwi-Minsker, Numbering-up and mass transfer studies of liquid-liquid two-phase microstructured reactors, Chem. Eng. J. 158 (2010) 233–240.

[17] S.K. Kurt, I. Vural Gürsel, V. Hessel, K.D.P. Nigam, N. Kockmann, Liquid-liquid extraction system with microstructured coiled flow inverter and other capillary setups for single-stage extraction applications, Chem. Eng. J. 284 (2016) 764–777.

[18] D. Liu, K. Wang, Y. Wang, Y. Wang, G. Luo, A simple online phase separator for the microfluidic mass transfer studies, Chem. Eng. J. 325 (2017) 342–349.

[19] F. Kaske, S. Dick, S.A. Pajoohi, D.W. Agar, The influence of operating conditions on the mass transfer performance of a micro capillary contactor with liquid–liquid slug flow, Chem. Eng. Process. Process Intensif. 108 (2016) 10–16.

[20] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702.

[21] N. Aoki, S. Tanigawa, K. Mae, A new index for precise design and advanced operation of mass transfer in slug flow, Chem. Eng. J. 167 (2011) 651–656.

[22] A. Matsuoka, K. Noishiki, K. Mae, Experimental study of the contribution of liquid film for liquid-liquid Taylor flow mass transfer in a microchannel, Chem. Eng. Sci. 155 (2016) 306–313.

[23] K.G. Biswas, S. Ray, G. Das, J.K. Basu, A simple flow device for enhanced mass transfer in reduced dimensions, Chem. Eng. J. 279 (2015) 973–982.

[24] M.N. Kashid, D.W. Agar, S. Turek, CFD modelling of mass transfer with and without chemical reaction in the liquid-liquid slug flow microreactor, Chem. Eng. Sci. 62 (2007) 5102–5109.

[25] M. Sattari-Najafabadi, M.N. Nasr Esfahany, Intensification of liquid-liquid mass transfer in a circular microchannel in the presence of sodium dodecyl sulfate, Chem. Eng. Process. Process Intensif. 117 (2017) 9–17.

[26] A.L. Dessimoz, L. Cavin, A. Renken, L. Kiwi-Minsker, Liquid-liquid two-phase flow patterns and mass transfer characteristics in rectangular glass microreactors, Chem. Eng. Sci. 63 (2008) 4035–4044.

[27] A. Woitalka, S. Kuhn, K.F. Jensen, Scalability of mass transfer in liquid-liquid flow, Chem. Eng. Sci. 116 (2014) 1–8.

[28] P. Plouffe, D.M. Roberge, A. Macchi, Liquid-liquid flow regimes and mass transfer in various micro-reactors, Chem. Eng. J. 300 (2016) 9–19.

[29] P. Plouffe, M. Bittel, J. Sieber, D.M. Roberge, A. Macchi, On the scale-up of micro-reactors for liquid-liquid reactions, Chem. Eng. Sci. 143 (2016) 216–225.

[30] A. Ghaini, M.N. Kashid, D.W. Agar, Effective interfacial area for mass transfer in the liquid-liquid slug flow capillary microreactors, Chem. Eng. Process. Process Intensif. 49 (2010) 358–366.

[31] N. Di Miceli Raimondi, L. Prat, C. Gourdon, P. Cognet, Direct numerical simulations of mass transfer in square microchannels for liquid-liquid slug flow, Chem. Eng. Sci. 63 (2008) 5522–5530.

[32] A.H.P. Skelland, R.M. Wellek, Resistance to mass transfer inside droplets, AIChE J. 10 (1964) 491–496.

[33] G. Berčič, A. Pintar, The role of gas bubbles and liquid slug lengths on mass transport in the Taylor flow through capillaries, Chem. Eng. Sci. 52 (1997) 3709–3719.

[34] J.M. van Baten, R. Krishna, CFD simulations of mass transfer from Taylor bubbles rising in circular capillaries, Chem. Eng. Sci. 59 (2004) 2535–2545.

[35] C.O. Vandu, H. Liu, R. Krishna, Mass transfer from Taylor bubbles rising in single capillaries, Chem. Eng. Sci. 60 (2005) 6430–6437.

[36] J. Yue, L. Luo, Y. Gonthier, G. Chen, Q. Yuan, An experimental study of air-water Taylor flow and mass transfer inside square microchannels, Chem. Eng. Sci. 64 (2009) 3697–3708.

[37] C. Mendoza, A theorem for rayleigh's method of dimensional analysis and its proof, Mech. Res. Commun. 21 (1994) 103–107.

[38] G.I. Taylor, Deposition of a viscous fluid on a plane surface, J. Fluid Mech. 9 (1961) 218.

[39] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71.

[40] M. Matsumoto, T. Takagi, K. Kondo, Separation of lactic acid using polymeric membrane containing a mobile carrier, J. Ferment. Bioeng. 85 (1998) 483–487.

[41] S.L. Oswal, R.G. Sindhe, A.T. Patel, J.P. Dave, S.G. Patel, B.M. Patel, Study of viscosity of mono-, di-, and trialkylamines, Int. J. Thermophys. 13 (1992) 617–628.

[42] D.R. Caudwell, J.P.M. Trusler, V. Vesovic, W.A. Wakeham, The viscosity and density of n-dodecane and n-octadecane at pressures up to 200 MPa and temperatures up to 473 K, Int. J. Thermophys. 25 (2004) 1339–1352.

[43] M.A. Saleh, S. Akhtar, S. Begum, M.S. Ahmed, S.K. Begum, Density and viscosity of 1-alkanols, Phys. Chem. Liq. 42 (2004) 615–623.

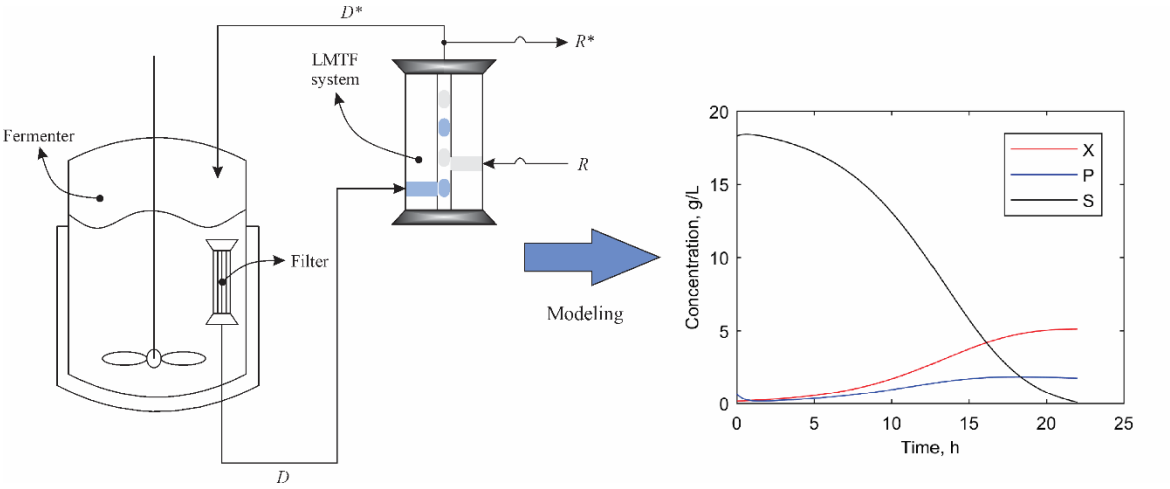
[44] A. Bahramian, A. Danesh, Prediction of liquid-liquid interfacial tension in multi-component systems, Fluid Phase Equilib. 221 (2004) 197–205.

[45] D. Villers, J.K. Platten, Temperature dependence of the interfacial tension between water and

long-chain alcohols, J. Phys. Chem. 92 (1988) 4023–4024.

[46] L. Arsenjuk, F. Kaske, J. Franzke, D.W. Agar, Experimental investigation of wall film renewal in liquid–liquid slug flow, Int. J. Multiph. Flow. 85 (2016) 177–185.

5. Chapter 5: Hybrid system



5.1 Integration of a liquid membrane in Taylor flow regime with a fermentation by *Lactobacillus casei* ATCC 393 for in-situ lactic acid removal[7]

Abstract

A new type of liquid membranes called liquid membrane in Taylor flow was integrated to a lactic acid fermentation, using *Lactobacillus casei* ATCC 393, for lactic acid removal during fermentation. The performance in terms of lactic acid production of the hybrid batch system is compared to a conventional batch fermentation. Lactic acid removal rate increases proportionally with the LA concentration within the fermenter. The lactic acid, the biomass production and the LA productivity in the hybrid system increased by 41.8, 12 and 26.6%, respectively, as compared to the conventional batch fermentation. However, toxicity effects reduce LA to glucose yield in 15.9% as compared to conventional fermentation. Liquid membranes in Taylor flow results promising for enhancing batch and continuous fermentation processes by a hybrid system.

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5.1.1 Introduction

Lactic acid fermentation

Lactic acid (LA) is a commodity chemical with significant applications in various fields [1,2] such as food (as additive and preservative), cosmetic and pharmaceutical industries [2–5]. Also, it is used as raw material of several chemicals, for instance, acrylic acid, propylene glycol, acetaldehyde, among others [1,2]. Additionally, LA can be used as a monomer for the production of polylactic acid (PLA) a biodegradable polymer [6,7].

LA can be produced by two routes, chemical synthesis or by carbohydrate fermentation [8–11]. Chemical synthesis produces the racemic mixture of the LA, while by fermentation route it can be produced pure L(+)-lactic acid [7,9,10,12] or pure D(-)-lactic acid, depending on the used microorganism [7]. D(-)-lactic acid is harmful to humans at high levels, whereas L(+)-lactic acid is assimilated by the human body [13]. Bacterial fermentation route offer advantages over chemical synthesis because it can be used cheap raw materials, low production temperatures, with low energy consumption and, it can be obtained one of the two isomers of LA [7,9,14].

Although 90% of the LA production is achieved through bacterial fermentation [10,13], the biotechnological route has used around 100 years without significant technological changes [15]. Batch processes have been the most commonly operation mode at industrial level [11,13]. Conventional recovery of LA from the fermentation broth is by precipitation, followed by filtration with the addition of sulfuric acid [2,15]. Subsequently, the LA is purified by activated carbon, evaporation and crystallization [1,15]. The conventional separation process for the production of LA is expensive and produces a significant amount of calcium sulfate (gypsum) as solid waste [1,2]. Some drawbacks to overcome in the conventional LA biotechnological production [15] are: Cell growth inhibition (by product and substrate), the use of neutralizers, low yield and productivity, and the high overall cost of the whole process [1,15]. The product inhibition of the lactic acid bacteria (LAB) is due to the acidic conditions affects the cytoplasm, the proton motive forces fail [5], and as consequence low concentrations of LA and biomass are obtained achieving low yields [2,4,12].

Lactic acid removal from the fermentation broth

In order to reduce the cost of the biotechnological LA process production, current studies have been addressed on the modifications of LAB to achieve acid-tolerant strains [1,11,15,16], on to obtain raw materials of low cost [11], and on the application of new separation technologies on fed-batch, semi-continuous and continuous fermentations [11]. However, since the cost of the separation and final purification steps of the conventional LA production process is around 50% of the total cost of

the process [1,2,15,17], several separation technologies for LA recovery from fermentation broth have attracted great attention in the recent years [2,3]. LA removal is also important from the point of view of product inhibition [18], because with the application of a suitable selective separation technique on the fermentation process it is possible to reduce the product inhibition effect [5,18]. The explored technologies are solvent and reactive extraction, adsorption, ion exchange, membrane separation, electrodialysis and reactive distillation [2,3,10,18,19]. One of the most studied separation technologies for LA removal has been reactive extraction [6,10,17,20–24]. However, this process requires high volumes of solvent and the use of back-extraction for regeneration of the solvent [5,10,25].

Liquid membrane separation is a membrane technology where the extractants and diluents of the reactive extraction can be used as a membrane phase [26]. In contrast to reactive extraction, it is not required the additional step of back-extraction, because both extraction and back-extraction occur simultaneously with a membrane process in a single unit [25,26]. Liquid membranes are a potential separation technology to be integrated with a fermentation process for in-situ LA removal which has been tested in several studies [4,11,17,27–32]. The liquid membrane in Taylor flow (LMTF) is a novel membrane technology that preserves the advantages of conventional emulsion liquid membranes while overcomes the stability problems of emulsion systems [33–35]. The LMTF has been developed and tested for LA removal [33–35].

This work analyses a hybrid system which involves the LMTF for in-situ LA removal from a batch LA fermentation (by *Lactobacillus casei* ATCC 393). The performance of the hybrid system is compared to a batch conventional fermentation in terms of LA productivity, yield and LA removal level.

5.1.2 Experimental

Strain and culture media

Fermentation broths were prepared at 10 g/L of tryptose (Scharlau), 20 g/L of dextrose anhydrous (Loba Chemie), 5 g/L of sodium acetate (anhydrous, Merck), 2 g/L of ammonium citrate dibasic (Sigma-Aldrich), 0.2 g/L of magnesium sulfate (Heptahydrate, Loba Chemie), 0.05 g/L of manganese sulfate (monohydrate, Loba Chemie), and 2 g/L of potassium phosphate dibasic (anhydrous, Loba Chemie). For each fermentation (batch and hybrid) it was prepared 70 mL of sterile fermentation broth with distilled water. The used lactic acid bacteria (LAB) for the fermentation was *Lactobacillus casei* ATCC 393 (Microbiologics). Pre-inoculum was prepared

using two cryogenized pearls of the LAB within 10 mL of MRS broth (Scharlau) into an incubator (RI 115, Binder) at 37 ºC. The inoculum was prepared at 37 ºC in 10 mL of the fermentation broth at 10 vol% of a culture of 24 h.

The experimental conditions and the fermentation broth for batch and hybrid fermentation systems were the same.

Fermentations

Fermentations were carried out at 37 ºC in a glass flask of 100 mL (GL 45, Duran®) and controlling the temperature with a water-bath using a digital hotplate stirrer (DAIHAN MaXtir™ 500) during 22 h. Samples of 0.3 mL were taken from the fermentation broth each two hours for lactic acid and glucose quantification by HPLC. One sample of 2 mL was taken at the beginning (0 h) and another sample at the end (22 h) of the fermentation for biomass quantification by dry cell weight method.

Fermentation volumes of 50 mL at 5 vol% of the inoculum were used to have a LA removal rate by the liquid membrane of the same order of magnitude that the LA production rate by the bacteria.

Liquid membrane in Taylor flow for lactic acid removal

The liquid membrane in Taylor flow (LMTF) is a new alternative of contact among the phases of a conventional liquid membrane (donor, membrane and receiving phases), which extends the Taylor flow to a three-phase system [33–35]. In LMTF the enhanced mass transfer is due to the features of the Taylor flow [36]. In the LMTF, the membrane phase (*M*) flows as a continuous phase within a channel while donor (*D*) and receiving (*R*) phases flows as a subsequent train of droplets (Figure 1a). The LA is transferred from the donor phase to the membrane phase and, from here, to the receiving phase. Donor and receiving phases are aqueous while the membrane is an organic phase (Figure 1b).

The injection of the dispersed phases (donor and receiving) is carried out by cycles. Each injection cycle follows the next four steps: First, the donor phase is injected during an injection time of the donor phase. Then, the injection of the donor phase is stopped and a delay time elapses. Afterward, the receiving phase starts to inject during an injection time of receiving phase, and in the fourth step, the receiving phase injection is stopped and a delay time elapses again [33,34]. At the outside of the channel, the receiving phase, rich of solute, and the donor phase, poor in solute, are fed to separate containers of a settler. In the containers of the settler the membrane phase is split from each dispersed phase. Then, the membrane phase is recycled to the LMTF system (Figure 1a).

The membrane phase is composed by tri-iso-octylamine (10 vol%), 1-dodecanol (40 vol%) and, n-dodecane (50 vol%), where the tertiary amine is the carrier (for facilitated transport), the alkane and the alcohol are the inert, and active diluents, respectively [34,37]. Tri-iso-octylamine (TiOA), 1-dodecanol, n-dodecane were supplied by Merck (all reagents for synthesis). This membrane phase was previously tested both by molecular toxicity on the *Lactobacillus casei* ATCC 393 [38] and by liquid-liquid equilibrium with LA aqueous solutions [37]. This membrane phase is able to remove LA while the molecular toxicity on the bacteria is relatively low [37].

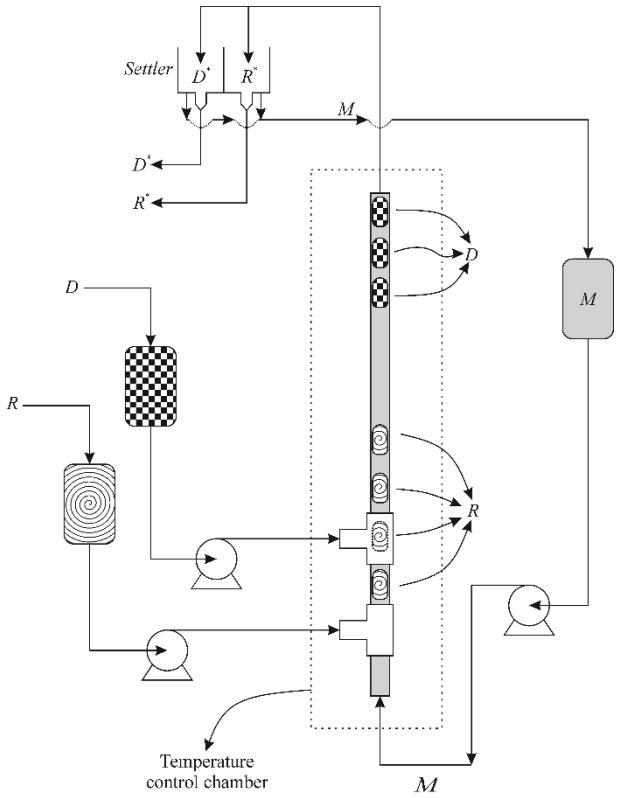

Figure 1a. Experimental set-up of the LMTF for solute removal [33,34]. *D*: Donor phase rich in the solute. *R*: Fresh receiving phase (free of solute). *M*: Membrane phase. D^*: Donor phase poor in the solute. R^*: Receiving phase rich in the solute.

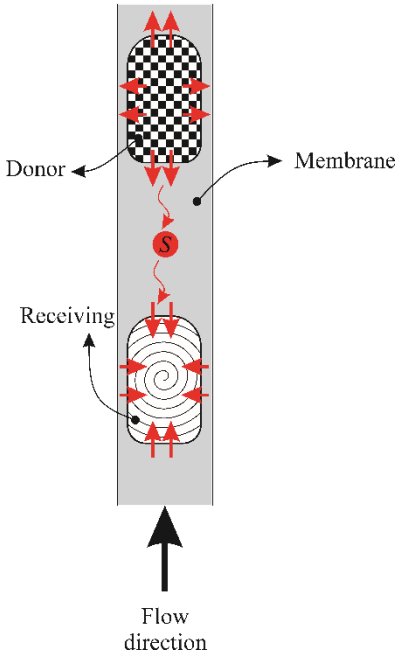

Figure 1b. Schematic solute (*S*) transport process between two adjacent droplets (donor and receiving) through the membrane phase for a LMTF system.

The donor phase is an aqueous solution which contains LA to remove or the fermentation broth free of biomass. The receiving phase is an aqueous solution of sodium carbonate (anhydrous, supplied by Merck) at 12 g/L prepared with water type I (Barnstead™ Nanopure™).

The injection flow rates of the involved phases within the LMTF were, 7 mL/min (by a HPLC pump, Waters 501) for membrane phase, 3.5 mL/min for donor phase (by a syringe pump, Cole-Parmer® Touch Screen Infuse/Withdraw) and 3.4 mL/min for receiving phase (by a gear pump Ismatec Reglo-z, coupled to a solenoid valve STNC® - DC 24V). The injection time and delay time were, 6 s and 18 s, respectively (for both donor and receiving phase). The involved phases were injected in a circular channel of 348.8 cm of length and 2.5 mm of inner diameter using a closed chamber with control temperature at 37 ºC [34,35].

Hybrid fermentation and LMTF system

In the hybrid system, the batch LA fermentation was coupled to the LMTF (Figure 2). The donor phase, which is fed to the LMTF, is the fermentation broth free of biomass from the fermenter. A hollow-fiber filter of 0.2 μm (Barnstead™) was used to retain biomass. When 10 mL of the culture media free of biomass is collected, it is fed to the LMTF. Then, the donor phase output stream from the LMTF is recirculated to the fermenter by gravity in a closed loop. Once the donor phase is returned to the fermenter from the LMTF system, another 10 mL of the fermentation broth is filtered and the process described above repeated till the end of the fermentation (20 h). Samples of 0.15 mL were taken both from donor and receiving output streams every 40 min for quantification of LA concentration by HPLC.

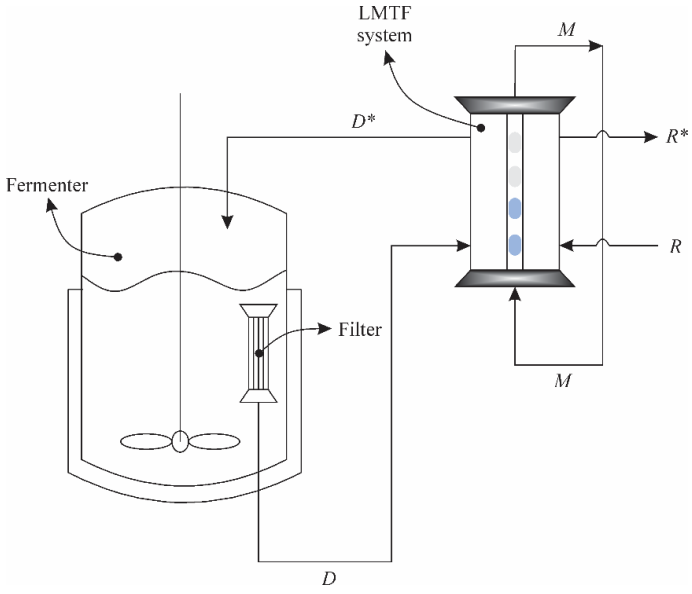

Figure 2. Scheme of the hybrid system (lactic acid fermentation integrated with the liquid membrane in Taylor flow). *D*: Donor phase input stream from the fermenter free of biomass. D^*: Donor phase output stream from the LMTF. *R*: Receiving phase input stream. R^*: Receiving phase output stream.

The efficiency of LA removal is calculated by Eq. (1) where $LA_{removed}$ is the mass of LA removed from donor phase in grams and LA_0 is the inlet LA mass in grams in the donor phase:

$$E(\%) = \frac{LA_{removed}}{LA_0} \times 100 \quad (1)$$

Analytical methods

Concentrations of LA and glucose were measured by HPLC (Hitachi LaChrom Elite®) with an ORH-801 column (Chrom Tech), an aqueous solution of 0.01 N of sulfuric acid for the mobile phase (at 0.8 mL/min), and a RI detector at 45 °C. For the preparation of the mobile phase, it was used sulfuric acid (for analysis) supplied by Merck. For calibration of glucose, it was used D(+)-glucose (anhydrous for biochemistry) supplied by Merck. The calibration of LA was prepared from a stock solution of LA at 150 g/L previously heated at 90 °C under total reflux between 8-10 hours for dimer hydrolysis [6,35,37]. The LA stock solution was prepared with L(+)-lactic acid supplied by Scharlau, which purity was assessed by titration with sodium hydroxide (Merck) using Metrohm automatic titrator (702 SM Titrino, 703 TI Stand). All aqueous solutions for calibration were prepared with water type I.

For biomass quantification, it was used Eppendorf tubes of 2 mL. The Eppendorf is dried in an oven (Binder) at 100 ºC during 36 h. Once the Eppendorf is dried, it is weighing on a precision scale (Dhaus) and then it is returned to the oven (at 100 ºC) for storage till the sample of the fermentation broth will be taken. Once the sample is taken from the fermentation broth, the Eppendorf is filled with 2 mL of sample (fermentation broth) and subsequently, the Eppendorf is centrifuged at 10000 rpm during 15 mins (Centrifuge 5415 C, Eppendorf). Afterward, the supernatant liquid of the centrifuged Eppendorf is discarded, the Eppendorf is filled with 1 mL of distilled water, agitated in a Bio vortex (Boeco) around 1 min and then it is centrifuged at the aforementioned conditions. Afterward, the supernatant liquid of the centrifuged Eppendorf is discarded and the Eppendorf is moved to the oven to be dried (at 100 ºC). The Eppendorf is weighing every 12 h till the weight of the Eppendorf will be constant.

5.1.3 Results and discussion

Improvements on the LA productivity and yield are expected when the LA is continuously removed from fermentation because the product inhibition effect is reduced [39]. The accumulation of LA

within the fermentation broth decreases the value of the pH (within the fermenter), and consequently, it produces the acidification of the cytoplasm and collapse of the proton motive forces affecting the nutrient transport [5,40]. At low values of pH within the fermentation broth, the concentration of the undissociated LA increases, which is cytoplasmic membrane soluble. Undissociated molecules of LA inside the cell affects the transmembrane pH which is no longer maintained and therefore disables the cellular functions [5]. Additionally, the energy consumption increases because the cell must increase its activity in order to maintain the transmembrane pH gradient and in consequence the cell growth is reduced [5]. On the other hand, it was shown that the decreasing of the cell growth is not only depending on the undissociated LA concentration but by the dissociated LA as well [40].

The carrier of the membrane used in this work is a tertiary amine that can react with bot dissociated (by ion pair) and undissociated (by H-bond) LA [37]. From this point of view, the inhibitory effect of both forms of LA can be reduced, through the LA removal in the LMTF.

In the hybrid system, the LA removal through the liquid membrane in Taylor flow (LMTF) started at 13.4 h and ends at 19.9 h. The glucose concentration in the hybrid system decreased faster than the batch system from the 13.4 h when the LA removal started (Figure 3). This effect can be due to glucose removal by the LMTF or glucose consumption increases due to the LA removal by the LMTF.

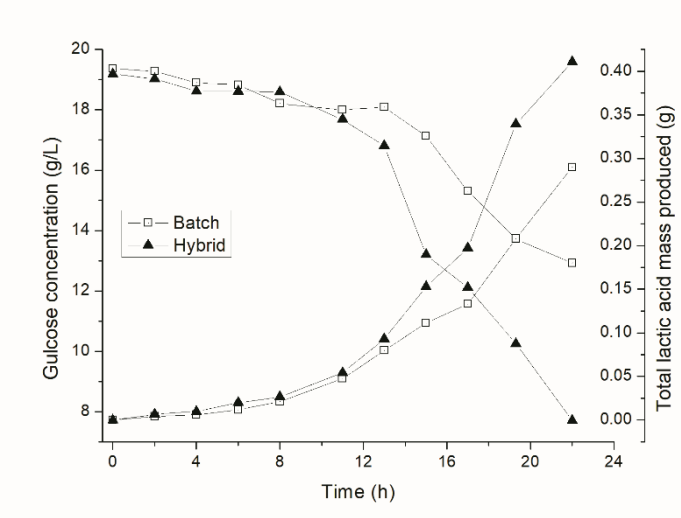

Figure 3. Glucose concentration within the fermenter for batch and hybrid systems and total LA mass produced in both systems.

The samples of the receiving phase showed that there was a mass transport of glucose through the LMTF, removing a total of 2.3 wt% of the total amount of initial glucose in the fermenter. However,

the average consumption rate of glucose from the 13 to 22 h in the hybrid system was 0.047 g/h while in the conventional fermentation was 0.029 g/h. Additionally, the LAB consumed 68.5% more glucose in the hybrid system than the batch system. Consequently, the amount of glucose removed by the LMTF is small as compared to the glucose consumption due to the fermentation process itself. The LA production is analyzed to study the increased glucose consumption of the hybrid system compared to the batch system.

The total LA mass produced is higher in the hybrid system than in the batch system from the moment that the LA removal started in the hybrid system until both fermentations ended (aprox. 13 h in Figure 3). The total LA mass produced in the hybrid system was 41.8% higher than the in batch system (Figure 3). The average production rate of LA (13 h to 22 h) was 0.023 and 0.035 g/h for batch and hybrid systems, respectively. The LA production was promoted in the hybrid system because a reduction of the inhibitory effect of the LA by removal through the LMTF. When LA is removed from the fermentation broth, the acidification of the LAB decreases and as consequence the inhibitory effect due to the fermentation concentration of product is reduced. Therefore, there is a better transport of nutrients toward the LAB, which contributes to the metabolic pathway in order to produce LA.

Since the glucose consumption increased 68.5% and LA production increased 41.8% in the hybrid system (compared to the batch system), it was expected an increase on biomass production around 26.7%. However, the total produced biomass was 0.625 and 0.7 g/L, in the batch and hybrid systems, respectively, that is an increase of 12%. Thus, based on these values there is a difference of 14.7% of glucose that is used for maintenance as a response to the molecular toxicity of the organic compounds in the membrane phase of the LMTF [38]. Previous studies of molecular toxicity of the membrane phase (TiOA/dodecanol/dodecane at 10, 40 and 50 vol%, respectively) on *Lactobacillus casei* ATCC 393 showed that glucose consumption is 18.9% higher than cell growth [38], which it is in agreement with the results of this work. Additionally, the time of contact and the way of contact between the membrane phase and donor phase (fermentation broth free of biomass) through the LMTF was different to those ones of the molecular toxicity test. In the LMTF, the time of contact was 6 h and in any point of time only a portion of the fermentation broth was in contact with the membrane phase, while in the molecular toxicity tests the time of contact was 72 h and the whole fermentation broth was in contact with the membrane phase in any point of time. Therefore, it is expected that the effect of the membrane phase on the fermentation broth keeps but in a lower

proportion compared to the molecular toxicity test. Hence, there is a slight difference between the glucose consumption between the LA removal through the LMTF and the molecular toxicity tests.

Table 1. Experimental productivity and yields of both fermentations (batch and hybrid) during 22 h of fermentation.

System	Productivity [g/(L·h)]	LA to glucose yield, [g LA/g glucose]	Biomass to glucose yield, [g biomass/g glucose]	LA to biomass yield, [g LA/g biomass]
Batch	0.2635	0.8994	0.0970	9.2736
Hybrid	0.3736	0.7567	0.0644	11.7410

It is observed that the LA to glucose and, biomass to glucose yields are higher in the batch system than the hybrid system (Table 1) because the higher glucose consumption in the hybrid system in relation with the batch system discussed above. On the other hand, the LA to biomass yield and the LA productivity are higher in the hybrid system than the batch system. The LA to biomass yield was 9.2736 and 11.7410 g LA/g biomass, while the productivity was 0.2635 and 0.3736 g/(L·h) in batch and hybrid systems, respectively.

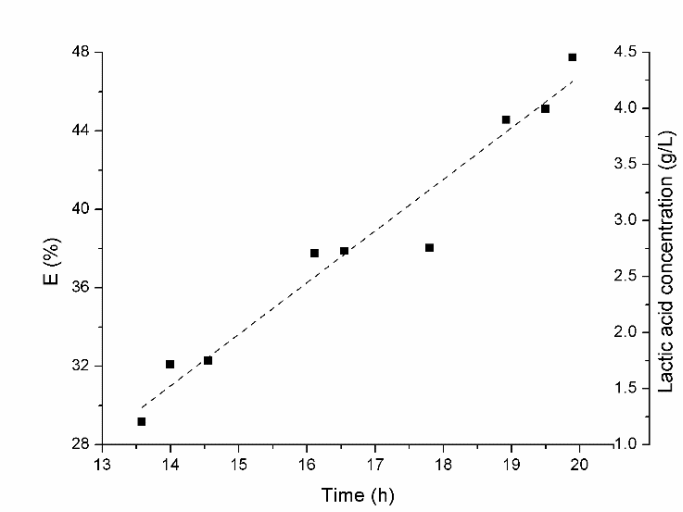

Figure 4. The efficiency of LA by Eq. (1) by the LMTF during the LA removal period (13.4 to 22 h), and the corresponding LA concentration within the fermenter.

The efficiency of LA removal in the LMTF is 29.2% at 13.4 h, when LA concentration within the fermenter is around 1.2 g/L (Figure 4). The corresponding efficiency at 19.9 h is 47.74%, with a LA concentration in the fermentation broth of 4.5 g/L. The efficiency of LA extraction increases as the LA concentration rises, as it was expected, because the driving force for LA mass transport between

the donor phase and the membrane phase increases. The LA concentration in the membrane phase is small because the LA is continuously removed from membrane phase by the receiving phase [33–35]. However, the LA removal through the LMTF not only depends on the driving force but the operational conditions of the LMTF as well, which directly affects the mass transfer coefficient [34,35]. However, the operating conditions were kept constant for the LMTF and thus the LA removal can change due to only changes in the LA driving force. As a consequence, Figure 4 shows that the slope of the corresponding curve is approximately constant which is related to the global mass transfer coefficient.

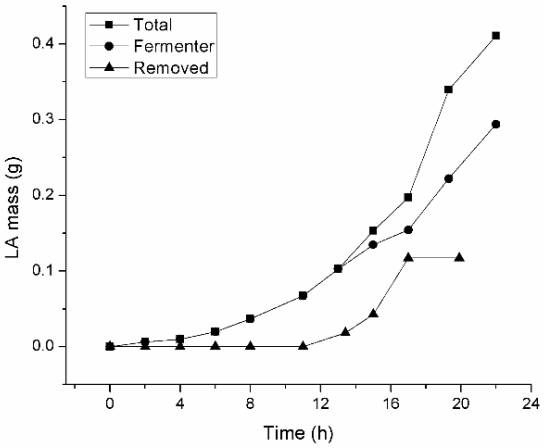

Figure 5. Mass of lactic acid in the hybrid system. Total produced (squares), within the fermenter (circles) and removed through the LMTF (triangles).

Figure 5 shows that the maximum amount of LA removed from the fermenter by the LMTF is 25% of the total amount of LA produced. Also, the slope of LA concentration in the fermenter is lower than the corresponding one for LA removal. Thus, at some point, that was not explored, the LA concentration in the fermenter will reach a maximum and it will decrease. The performance of the hybrid system can be improved by increasing the amount of LA removed by the liquid membrane. The following alternatives can be explored as compared to the conditions used in this study: a) Increasing the volumetric flows of donor phase to the LMTF. b) Increasing the number of channels of the LMTF. The volumetric flow of the donor phase in a single channel cannot be increased at will because the Taylor regime has to be produced [41–44]. If the number of channels in the LMTF is increased, it is expected that the LA and biomass productivity will increase with the same level of

molecular toxicity shown in this study. Molecular toxicity does not depend on interfacial area but on the amount of organics dissolved in the fermentation broth.

5.1.4 Conclusions

A hybrid system of fermentation for lactic acid (LA) production with *Lactobacillus casei* ATCC 393 and LA removal by a liquid membrane in Taylor flow was experimentally tested and compared to a conventional fermentation. The hybrid batch process shows a LA and biomass production of 41.8% and 12% higher than the convention batch fermentation, respectively. Consequently, LA productivity of the hybrid process is 25.8% higher than the conventional fermentation.

However, the glucose consumption of the hybrid system was 68.5% higher than in conventional fermentation. In the hybrid system, the glucose intake of the biomass is partially (12%) used for maintenance as a response to molecular toxicity and thus, the LA to glucose yield is lower (15.9%) than in a conventional fermentation.

The LA extraction efficiency of the LMTF system increases as the LA concentration of the fermentation broth rises. This efficiency can be improved if several channels are used in the LMTF.

The LMTF can be integrated with fermentation processes to remove metabolites and enhance both LA and biomass productivity, however molecular toxicity issues could reduce LA to glucose yield.

5.1.5 References

[1] M. Singhvi, T. Zendo, K. Sonomoto, Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects, Appl. Microbiol. Biotechnol. 102 (2018) 5911–5924. doi:10.1007/s00253-018-9092-4.

[2] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[3] N. Phanthumchinda, S. Thitiprasert, S. Tanasupawat, S. Assabumrungrat, N. Thongchul, Process and cost modeling of lactic acid recovery from fermentation broths by membrane-based process, Process Biochem. 68 (2018) 205–213. doi:10.1016/j.procbio.2018.02.013.

[4] M. Othman, A.B. Ariff, H. Wasoh, M.R. Kapri, M. Halim, Strategies for improving production performance of probiotic Pediococcus acidilactici viable cell by overcoming

lactic acid inhibition, AMB Express. 7 (2017). doi:10.1186/s13568-017-0519-6.

[5] M. Othman, A.B. Ariff, L. Rios-Solis, M. Halim, Extractive Fermentation of Lactic Acid in Lactic Acid Bacteria Cultivation: A Review, Front. Microbiol. 8 (2017) 1–7. doi:10.3389/fmicb.2017.02285.

[6] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria for Trioctylamine/1-Dodecanol/Lactic Acid/Water System at 306.1, 310.1 and 316.1 K: Experimental Data and Prediction, J. Chem. Eng. Data. 61 (2016) 2269–2276. doi: 10.1021/acs.jced.5b00955.

[7] M.A. Abdel-Rahman, Y. Tashiro, K. Sonomoto, Recent advances in lactic acid production by microbial fermentation processes., Biotechnol. Adv. 31 (2013) 877–902. doi:10.1016/j.biotechadv.2013.04.002.

[8] P. Pal, J. Sikder, S. Roy, L. Giorno, Process intensification in lactic acid production: A review of membrane based processes, Chem. Eng. Process. Process Intensif. 48 (2009) 1549–1559. doi.org/10.1016/j.cep.2009.09.003.

[9] T. Ghaffar, M. Irshad, Z. Anwar, T. Aqil, Z. Zulifqar, A. Tariq, M. Kamran, N. Ehsan, S. Mehmood, Recent trends in lactic acid biotechnology: A brief review on production to purification, J. Radiat. Res. Appl. Sci. 7 (2014) 222–229. doi:10.1016/j.jrras.2014.03.002.

[10] A. Komesu, M.R. Wolf Maciel, R. Maciel Filho, Separation and Purification Technologies for Lactic Acid – A Brief Review, BioResources. 12 (2017) 6885–6901. doi:10.15376/biores.12.3.6885-6901.

[11] C. Rodrigues, L.P.S. Vandenberghe, A.L. Woiciechowski, J. de Oliveira, L.A.J. Letti, C.R. Soccol, Production and Application of Lactic Acid, in: Curr. Dev. Biotechnol. Bioeng., Elsevier, 2017: pp. 543–556. doi:10.1016/B978-0-444-63662-1.00024-5.

[12] M.A. Abdel-Rahman, K. Sonomoto, Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid, J. Biotechnol. 236 (2016) 176–192. doi:10.1016/j.jbiotec.2016.08.008.

[13] J. Vijayakumar, R. Aravindand, T. Viruthagiri, Recent Trends in the Production, Purification and Application of Lactic Acid, Chem. Biochem. Eng. Q. 22 (2008) 245–264.

[14] M. Boonmee, O. Cotano, S. Amnuaypanich, N. Grisadanurak, Improved Lactic Acid Production by In Situ Removal of Lactic Acid During Fermentation and a Proposed Scheme

for Its Recovery, Arab. J. Sci. Eng. 41 (2016) 2067–2075. doi:10.1007/s13369-015-1824-5.

[15] C. Miller, A. Fosmer, B. Rush, T. McMullin, D. Beacom, P. Suominen, Industrial Production of Lactic Acid, in: Ref. Modul. Life Sci., Elsevier, 2017: pp. 179–188. doi:10.1016/B978-0-12-809633-8.09142-1.

[16] I. Eş, A. Mousavi Khaneghah, F.J. Barba, J.A. Saraiva, A.S. Sant'Ana, S.M.B. Hashemi, Recent advancements in lactic acid production - a review, Food Res. Int. 107 (2018) 763–770. doi:10.1016/j.foodres.2018.01.001.

[17] N. Tik, E. Bayraktar, Ül. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, J. Chem. Technol. Biotechnol. 76 (2001) 764–768. doi.org/10.1002/jctb.449.

[18] E. Cubas-Cano, C. González-Fernández, M. Ballesteros, E. Tomás-Pejó, Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate, Biofuels, Bioprod. Biorefining. 12 (2018) 290–303. doi:10.1002/bbb.1852.

[19] R. Alves de Oliveira, A. Komesu, C.E. Vaz Rossell, R. Maciel Filho, Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects, Biochem. Eng. J. 133 (2018) 219–239. doi:10.1016/j.bej.2018.03.003.

[20] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702. doi:10.1021/acs.iecr.5b04917.

[21] A. Krzyzaniak, B. Schuur, A.B. De Haan, Equilibrium studies on lactic acid extraction with N,N-didodecylpyridin-4-amine (DDAP) extractant, Chem. Eng. Sci. 109 (2014) 236–243. doi.org/10.1016/j.ces.2014.01.030.

[22] A. Krzyzaniak, M. Leeman, F. Vossebeld, T.J. Visser, B. Schuur, A.B. De Haan, Novel extractants for the recovery of fermentation derived lactic acid, Sep. Purif. Technol. 111 (2013) 82–89. doi.org/10.1016/j.seppur.2013.03.031.

[23] D. Yankov, J. Molinier, J. Albet, G. Malmary, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri-n-octylamine dissolved in decanol and dodecane, Biochem. Eng. J. 21 (2004) 63–71. doi.org/10.1016/j.bej.2004.03.006.

[24] R. Juang, R. Huang, Equilibrium studies on reactive extraction of lactic acid with an amine

extractant, Chem. Eng. J. 65 (1997) 47–53. doi.org/10.1016/S0923-0467(97)03117-5.

[25] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications, 3rd ed., Elsevier, Amsterdan, 2003.

[26] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[27] C. Schöller, J.B. Chaudhuri, D.L. Pyle, Emulsion liquid membrane extraction of lactic acid from aqueous solutions and fermentation broth, Biotechnol. Bioeng. 42 (1993) 50–58. doi:10.1002/bit.260420108.

[28] R. Juang, S. Lee, R. Shiau, Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes, J. Memb. Sci. 137 (1997) 231–239. doi.org/10.1016/S0376-7388(97)00206-8.

[29] S. Schlosser, E. Sabolová, J. Marták, Pertraction and membrane based solvent extraction of carboxylic acids in hollow fiber contactors, in: Solvent Extr. 21st Century, Society of Chemical Industry, 2001: pp. 1041–1046.

[30] N. Rastogi, B.S. Chanukya, Supported Liquid Membrane Composed of Tertiary or/and Quaternary Amine for the Extraction of Lactic Acid, Int. J. Membr. Sci. Technol. 2 (2015) 19–28. doi:10.15379/2410-1869.2015.02.02.03.

[31] F. Garavand, S.H. Razavi, I. Cacciotti, Synchronized extraction and purification of L-lactic acid from fermentation broth by emulsion liquid membrane technique, J. Dispers. Sci. Technol. 39 (2018) 1291–1299. doi:10.1080/01932691.2017.1396225.

[32] A. Kumar, A. Thakur, P.S. Panesar, Lactic acid extraction using environmentally benign Green emulsion ionic liquid membrane, J. Clean. Prod. 181 (2018) 574–583. doi:10.1016/j.jclepro.2018.01.263.

[33] J. Fontalvo, A.D. Pérez, Membrana Líquida y proceso para realizarlo, Rad. 15-131023, n.d.

[34] A.D. Pérez, J. Fontalvo, A new concept of liquid membranes in Taylor flow: performance for lactic acid removal, Chem. Eng. Process. - Process Intensif. *submited* (2019).

[35] A.D. Pérez, B. Van der Bruggen, J. Fontalvo, Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation, Chem. Eng. Process. -

Process Intensif. 134 (2018) 20–27. doi.org/10.1016/j.cep.2018.10.010.

[36] Y. Okubo, T. Maki, N. Aoki, T. Hong Khoo, Y. Ohmukai, K. Mae, Liquid-liquid extraction for efficient synthesis and separation by utilizing micro spaces, Chem. Eng. Sci. 63 (2008) 4070–4077. doi:10.1016/j.ces.2008.05.017.

[37] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Liquid–Liquid Equilibria of Lactic Acid/Water Solutions in Tri-iso-octylamine/Dodecane/1-Dodecanol at 306.1, 310.1, and 316.1 K. Experimental Data and Prediction, J. Chem. Eng. Data. submited (2019) acs.jced.8b00794. doi:10.1021/acs.jced.8b00794.

[38] A.D. Pérez, V.M. Gómez, S. Rodríguez-Barona, J. Fontalvo, Liquid-liquid Equilibrium and Molecular Toxicity of Active and Inert diluents of the Organic Mixture Tri-iso-octylamine/Dodecanol/Dodecane as Potential Membrane Phase for Lactic Acid Removal, J. Chem. Eng. Data. *submited* (2019).

[39] G. Burgé, M. Moussa, C. Saulou-Bérion, F. Chemarin, M. Kniest, F. Allais, H.-E. Spinnler, V. Athès, Towards an extractive bioconversion of 3-hydroxypropionic acid: study of inhibition phenomena, J. Chem. Technol. Biotechnol. 92 (2017) 2425–2432. doi:10.1002/jctb.5253.

[40] L.M.D. Gonçalves, A. Ramos, J.S. Almeida, A.M.R.B. Xavier, M.J.T. Carrondo, Elucidation of the mechanism of lactic acid growth inhibition and production in batch cultures of Lactobacillus rhamnosus, Appl. Microbiol. Biotechnol. 48 (1997) 346–350. doi:10.1007/s002530051060.

[41] J. Jovanović, W. Zhou, E. V. Rebrov, T.A. Nijhuis, V. Hessel, J.C. Schouten, Liquid-liquid slug flow: Hydrodynamics and pressure drop, Chem. Eng. Sci. 66 (2011) 42–54. doi:10.1016/j.ces.2010.09.040.

[42] Z. Cao, Z. Wu, B. Sundén, Dimensionless analysis on liquid-liquid flow patterns and scaling law on slug hydrodynamics in cross-junction microchannels, Chem. Eng. J. 344 (2018) 604–615. doi:10.1016/j.cej.2018.03.119.

[43] K. Zhang, Y.D. Hou, W.X. Tian, Y.P. Zhang, G.H. Su, S.Z. Qiu, Flow pattern effect on two-phase pressure drops in vertical upward flow across a horizontal tube bundle, Ann. Nucl. Energy. 120 (2018) 253–264. doi:10.1016/j.anucene.2018.05.059.

[44] A.A. Yagodnitsyna, A. V. Kovalev, A. V. Bilsky, Flow patterns of immiscible liquid-liquid

flow in a rectangular microchannel with T-junction, Chem. Eng. J. 303 (2016) 547–554. doi:10.1016/j.cej.2016.06.023.

5.2 Modeling of a liquid membrane in Taylor flow integrated with lactic acid fermentation[8]

Abstract

The application of a liquid membrane in Taylor flow (LMTF) is a promising method that can be integrated with other separation or reactive processes in view of process intensification. In this work, a model for a hybrid LMTF – fermentation system was developed for lactic acid production using batch fermentation and LMTF experimental data. The hybrid model is compared to experimental data of the hybrid system. Through a sensitivity analysis of the main variables of the LMTF an optimum value of the overall volumetric mass transfer coefficient (0.0122 1/s) was achieved for lactic acid removal. This was further used for modeling the hybrid system. The fermentation time of the hybrid system is reduced by 7 h, the productivity and biomass concentration is increased by 2.578 g/(L·h) and 2.7016 g/L, respectively, as compared with a batch fermentation. In addition, the effect of the number of channels of the LMTF is modeled and its impact on productivity, fermentation time, and final biomass concentration is analyzed. It was concluded that lactic acid removal through the LMTF from the fermentation broth is an alternative to control the pH within fermenter.

[8] This section has been submitted for publication: Alan D. Pérez, Bart Van der Bruggen, Javier Fontalvo (2019).

5.2.1 Introduction

Perstraction is a separation process that involves extraction and back extraction combined with membrane separation in a single unit [1,2]. Liquid membranes are used in perstraction processes, and have attracted the attention of engineers and scientists because of their advantages over liquid-liquid extraction and solid membrane processes [1]. Currently, a liquid membrane in Taylor flow (LMTF) has been proposed as an alternative for contacting the phases of liquid membranes (LM) to overcome the stability problems of the conventional liquid membranes, while keeping high mass transfer rates [3,4]. An LMTF is an advanced technique for recovery, purification, and abatement of substances that can be integrated with other separation or reactive fermentative-processes [1] to increase the performance and the productivity of conversion [5]. An LMTF has been tested for lactic acid (LA) removal [3,4], using a membrane phase with low toxicity levels for *Lactobacillus casei* ATCC 393 (a probiotic lactic acid bacteria) and a good capacity for LA extraction [6,7]. A model to calculate the overall volumetric mass transfer coefficients (OVMTC) for transport of LA through the phases of the LMTF has been proposed based on experimental data [4].

The production of lactic acid (LA) by the biotechnological route, which corresponds to 90% of the total production, has around 100 years of history without significant technological changes [8–10]. The corresponding cost of separation and final purification of LA is around 50% of the total cost of the process [10–13]. This fermentative process is interesting for its integration to LMTF for *in situ* removal of LA, reducing the known drawbacks of the fermentative process such as LA inhibition. The LMTF has been experimentally integrated to LA fermentation by *Lactobacillus casei* ATCC 393, as a hybrid system [14]. The LMTF used in the aforementioned hybrid process has 1 channel of 348.8 cm length and 2.5 mm inner diameter. By using this single channel LMTF , the LA productivity and biomass production were increased by 41.8 and 12%, respectively [14]. The impact of a LMTF on the batch LA fermentation can be higher if a multi-channel LMTF is used to increase the amount of fermentation broth processed through the LMTF.

In this work, a sensitivity analysis of the OVMTC of the LMTF for LA removal was carried out by using the model proposed in a previous paper [4]. This study shows the impact of operating conditions on the mass transfer through the LMTF, and presents a set of operating conditions that maximize the LA mass transfer in the LMTF. Then, a model for the hybrid LMTF – fermentation system, which includes a multi-channel LMTF, is developed. The model of the hybrid system developed here is compared with accepted experimental data [14]. Through the model, the impact of the number of channels in the LMTF on the LA productivity and biomass production is analyzed.

5.2.2 Theoretical

Liquid membrane in Taylor flow

A liquid membrane in Taylor flow (LMTF) is a new liquid membrane process, which extends the Taylor flow regime to a three-phase system [3,15], taking advantage of mass transfer features of this multiphase flow [16]. Perez A.D., *et al*. [3,4] show that a LMTF consists of a continuous membrane phase (slugs) that flows in a channel or tubing, while it is injected, in an alternating sequence, trains of droplets of a donor and a receiving phases (Figure 1). The solute is transported from donor droplets to membrane phase, and from here to receiving phases, through the respective interphases.

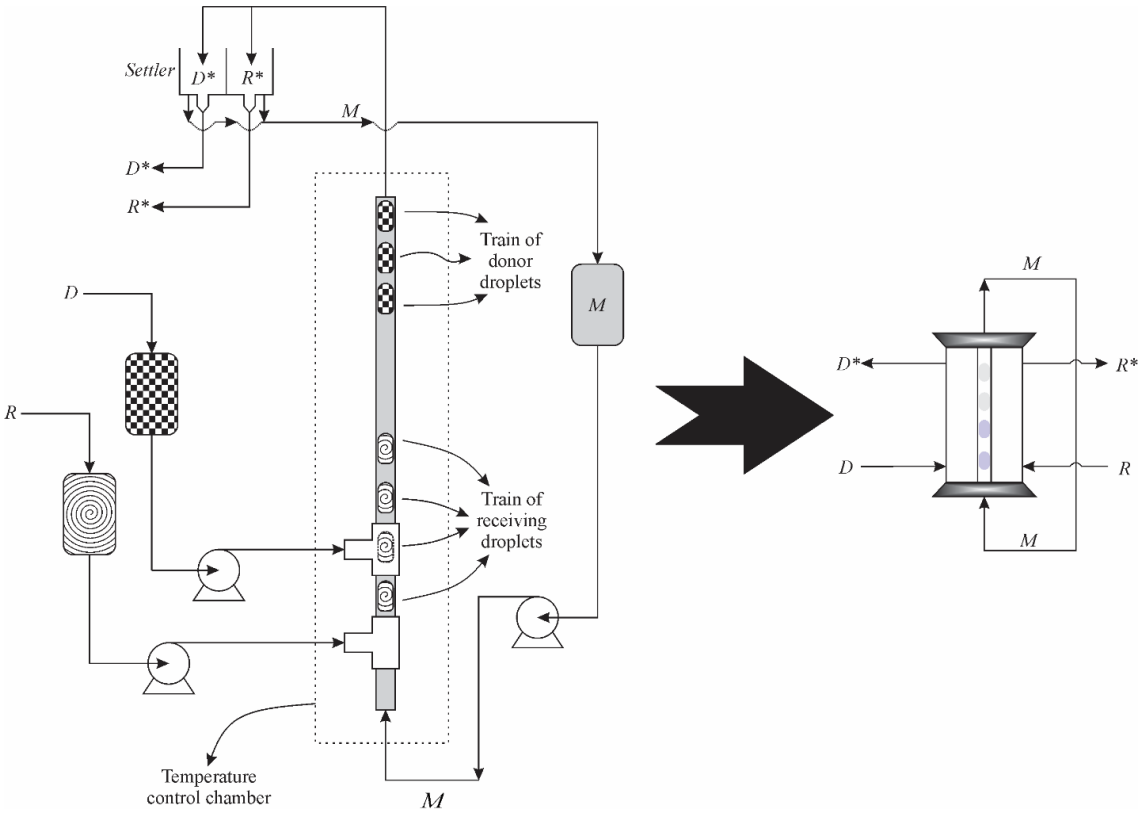

Figure 1. Experimental set-up (left) and scheme (right) of the LMTF system. *D*: Donor phase rich in LA. *R*: Fresh receiving phase. D^*: Donor phase poor in LA. R^*: Receiving phase loaded of LA. *M*: Membrane phase.

Overall volumetric mass transfer coefficients (OVMTC) at several experimental conditions have been calculated for transport of LA through the LMTF (from donor to membrane and from membrane to receiving) using a membrane phase that contains a carrier [4]. The flux of solute (in this case, LA) transported from donor droplets to the membrane phase is described by Eq. (1).

$$J_D = \frac{dC_D}{dt} = k_{L,D} a_D \left(C_D^{eq} - C_D \right) \tag{1}$$

where C_D is the concentration of LA (the solute) in any space-time of the channel of the LMTF, C_D^{eq} is the LA equilibrium concentration and $k_{L,D}a_D$ is the overall volumetric mass transfer coefficient from the donor to the membrane phases.

LA removal was experimentally tested by using a LMTF [3] with the membrane phase composed of tri-iso-octylamine (carrier) and two diluents, dodecane and dodecanol which are inert and active diluents, respectively. The tertiary amine reacts instantaneously and reversibly with the LA in the interphase donor/membrane and transports the produced complex through the membrane phase to the receiving phase, where the LA is released [3,4].

In systems where a carrier is involved in the membrane phase, the LA concentration in equilibrium (C_D^{eq}) can be assumed zero, because the carrier instantaneously reacts with the LA that comes from donor phase, producing a complex [17–20]. The integrated form of the flux of LA including the aforementioned assumption is as follows:

$$C_D(\tau) = \exp(-k_{L,D} a_D) \cdot C_D(\tau = 0) \tag{2}$$

For calculation of the OVMTC of Eq. (2), a semi-empirical model was developed that takes the main variables that affect the mass transport through the LMTF into account, and expresses some important physical properties of the Taylor flow regime as dimensionless numbers [4]:

$$k_{L,D} a_D = \alpha_1 \cdot \left(\frac{U_{T,D}}{d} \right) \cdot \left(\mathrm{Re}_D \right)^{\alpha_2} \cdot \left(Ca_D \right)^{-\alpha_3} \cdot \left(1 - W_D \right)^{\alpha_4} \cdot \left(\frac{U_{T,D} \cdot t_{inj}}{d} \right)^{\alpha_5} \cdot \left(\frac{L_{ch}}{d} \right)^{\alpha_6} \cdot \left(\frac{L_{slug}}{d} \right)^{\alpha_7} \tag{3}$$

The parameters of Eq. (3) can be fitted with experimental results [3]. Once the fitted parameters (α_i) are known, a sensitivity analysis of the OVMTC in Eq. (3) is performed by varying Ca_D, W_D, Re_D (which involves donor and membrane phase velocities), injection time of donor droplets (t_{inj}) and slug length (L_{slug}). From this sensitivity analysis it is possible to establish a range of operating

conditions that provide an optimal OVMTC for LA removal. Additionally, the effect of the space-time (τ) is modeled at each studied [3] experimental condition to find a range of space-times at a fixed OVMTC that provides the maximum LA transfer from the donor phase within the range of evaluated variables (Table 1).

Table 1. Range of each variable used in the sensitivity analysis for the OVMTC with Eq. (3) with a channel length of 348.8 cm and an inner channel diameter of 2.5 mm.

Variable	Range
L_{slug}	1 – 45 cm
t_{inj}	2.73 – 7.56 s
Q_D	0.0091 – 0.1259 cm^3/s
W_D	0.1111 – 0.3077
Re_D	6.7498 – 33.7491
Ca_D	0.0027 – 0.0136

Kinetic model for lactic acid fermentation by *Lactobacillus casei* ATCC 393

The kinetic model proposed by Monteagudo *et al*. [21] provides an adequate description of the concentration profiles for lactic acid fermentation. The kinetic model represents cell growth, lactic acid production and sugar consumption by three rate equations, taking inhibitory parameters both for the LA production and for cell growth due to the product concentration into account.

The specific growth rate, Eq. (4), is a function of the substrate concentration by a Monod equation:

$$\mu = \mu_{\max}\left(\frac{S}{K_S + S}\right) \tag{4}$$

For biomass production, Eq. (5) is used to predict cell growth as the product concentration rises until an inhibitory product concentration ($P_{\max}$), where the bacteria do not grow anymore.

$$\frac{dX}{dt} = \mu \cdot X\left(1 - \frac{P}{P_{\max}}\right) \tag{5}$$

The product rate, Eq. (6), is a function of biomass concentration including product formation by growth-associated and non-growth associated contributions. This model also includes product inhibition by $P'_{\max}$, which is the LA concentration where the bacteria cease to produce LA.

$$\frac{dP}{dt} = \left(A\frac{dX}{dt} + B \cdot X \right) \cdot \left(1 - \frac{P}{P'_{max}} \right) \tag{6}$$

Substrate utilization, described by Eq. (7), includes the stoichiometric substrate conversion in biomass ($Y_{X/S}$) and product ($Y_{P/S}$) and, the substrate consumption for maintenance (m), which is generally first-order with respect to biomass concentration.

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \cdot \frac{dX}{dt} - \frac{1}{Y_{P/S}} \cdot \frac{dP}{dt} - m \cdot X \tag{7}$$

The kinetic parameters were fitted through an in-house routine developed in Matlab® using the non-linear least square method and the function *globalsearch*. The minimization function is described by Eq. (8):

$$f_{min} = \sum_{i=1}^{n} \left[\left(X_i^{calc} - X_i^{exp} \right)^2 + \left(P_i^{calc} - P_i^{exp} \right)^2 + \left(S_i^{calc} - S_i^{exp} \right)^2 \right] \tag{8}$$

In the minimization equation, the least squares between the experimental (n, is the number of experimental data) was used to calculate values of biomass, product, and substrate.

Development of the model for the integrated fermentation-separation system

The proposed hybrid system comprises a batch lactic acid fermentation integrated to a LMTF for LA removal during the fermentation process (Figure 2).

The material balances for the hybrid system were developed through the control volume shown in Figure 2 (discontinuous line), taking into the account the change of volume within the fermenter (dV_L/dt) due to the outgoing and ingoing streams. For material balances, the reaction rates are taken from the batch model described in the section of the LA kinetics.

The balance for the biomass is described in Eq. (9) with μ as shown in Eq. (4).

$$\frac{dX}{dt} = \mu \cdot X \left(1 - \frac{P}{P_{max}} \right) - \frac{X}{V_L} \cdot \frac{dV_L}{dt} \tag{9}$$

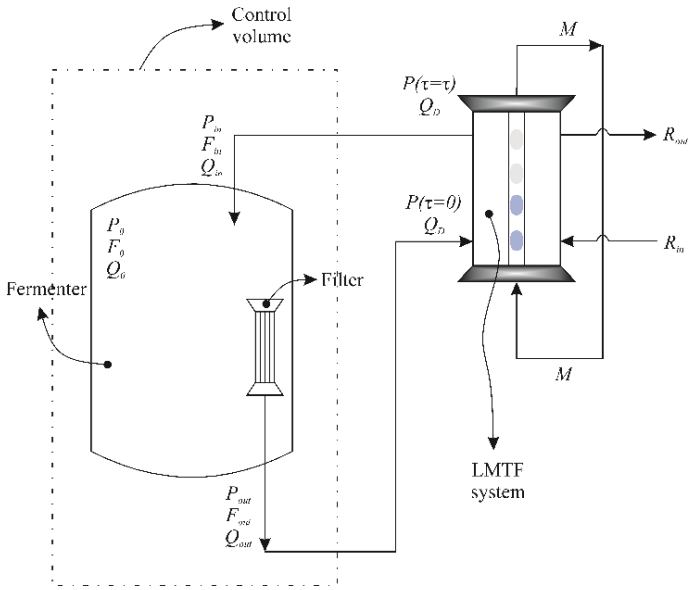

Figure 2. Scheme of the LMTF integrated into a fermenter for product removal including the control volume for material balances.

For the material balance of the lactic acid (product), there is an LA out-stream (F_{out}) which is processed by the LMTF system (Figure 2). Therefore, the in-stream (F_{in}) to the fermenter is a stream of low LA concentration. The product balance is described by Eq. (10) as follows:

$$\frac{dP}{dt} = \left(A\frac{dX}{dt} + B \cdot X \right) \cdot \left(1 - \frac{P}{P'_{\max}} \right) - \frac{P}{V_L} \cdot \frac{dV_L}{dt} - \frac{F_{out}}{V_L} + \frac{F_{in}}{V_L} \tag{10}$$

The material balance for the substrate in the hybrid system, including the change of volume within the fermenter is as follows:

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \cdot \frac{dX}{dt} - \frac{1}{Y_{P/S}} \cdot \frac{dP}{dt} - m \cdot X - \frac{S}{V_L} \cdot \frac{dV_L}{dt} \tag{11}$$

For the volume change within the fermenter, a mass flow balance is developed assuming constant density into the fermenter.

$$\frac{dm}{dt} = \dot{m}_{in} - \dot{m}_{out} \tag{12}$$

$$\frac{d(\rho \cdot V)}{dt} = \rho \cdot \frac{dV}{dt} = \dot{m}_{in} - \dot{m}_{out} \tag{13}$$

$$\frac{dV_L}{dt} = Q_{in} - Q_{out} \tag{14}$$

Q_D can be assumed constant because this stream has a high water concentration, which makes the change in volume due to LA removal negligible. Therefore, for simplification of the model for the hybrid system, it can be assumed that the inlet volumetric flow of donor phase is the same as the outlet volumetric flow of donor phase. Hence, Eq. (14) is equal to zero, and the model of the hybrid system is reduced to:

$$\frac{dX}{dt} = \mu \cdot X\left(1 - \frac{P}{P_{\max}}\right) \tag{15}$$

$$\frac{dP}{dt} = \left(A\frac{dX}{dt} + B \cdot X\right) \cdot \left(1 - \frac{P}{P'_{\max}}\right) - \frac{F_{out}}{V_L} + \frac{F_{in}}{V_L} \tag{16}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \cdot \frac{dX}{dt} - \frac{1}{Y_{P/S}} \cdot \frac{dP}{dt} - m \cdot X \tag{17}$$

From the control volume, three common variables can be identified for the shared streams between the fermenter and the LMTF system.

$$Q_{out} = Q_D = Q_{in} \tag{18}$$

$$P_{out} = P(\tau = 0) \tag{19}$$

$$P_{in} = P(\tau = \tau) \tag{20}$$

For calculation of the LA concentration in the stream that goes from the LMTF to the fermenter, the model of the LMTF (Eq. (2)) is used. The OVMTC and the space-time are depending on the operating conditions of the LMTF system.

$$P_{in} = \exp(-k_{L,D}a_D \cdot \tau) \cdot P_{out} \tag{21}$$

In Eq. (21) the outlet LA concentration from the fermenter (P_{out}) is the LA concentration at any point of time within the fermenter provided by Eq. (16). The inlet and outlet mass flows of LA from the fermenter are calculated through Eqs. (22) and (23), which involves the respective LA concentration and the volumetric flow of the donor phase through the LMTF.

$$F_{out} = P_{out} \cdot Q_D \quad (22)$$

$$F_{in} = P_{in} \cdot Q_D \quad (23)$$

In order to increase the capacity of the LMTF, a multi-channel LMTF system is proposed. The number of channels (N_{ch}) within the LMTF system is included in Eqs. (22) and (23), yielding the following equations:

$$F_{out} = P_{out} \cdot Q_D \cdot N_{ch} \quad (22b)$$

$$F_{in} = P_{in} \cdot Q_D \cdot N_{ch} \quad (23b)$$

The hybrid system was modeled at the same initial conditions of the batch fermentation with a LMTF system with one channel in order to compare both systems (batch and hybrid). For the LMTF the operating conditions were selected that provide the maximum value of OVMTC with a suitable space-time value. Then, the effect of the number of channels of the LMTF on the productivity, the total mass of LA achieved and final biomass concentration was explored. Additionally, the effect of the operating conditions of the LMTF (through the OVMTC) and the number of channels on the final pH achieved within the fermenter was studied.

5.2.3 Experimental

Lactic acid batch fermentation by *Lactobacillus casei* ATCC 393

Fermentation was carried out with the lactic acid bacteria (LAB) *Lactobacillus casei* ATCC 393 (Microbiologics), and molecular toxicity of the components of the membrane phase on the LAB was tested [6,7]. Pre-inoculum was prepared using two cryogenized pearls of the LAB within 25 mL of MRS broth (Scharlau) into an incubator (RI 115, Binder) at 37 ºC. The inoculum was prepared at 37 ºC in 25 mL of the fermentation broth at 10 vol% of a culture of 24 h. The fermentation broth was prepared at 10 g/L of tryptose (Scharlau), 20 g/L of dextrose anhydrous (Loba Chemie), 5 g/L of sodium acetate (anhydrous, Merck), 2 g/L of ammonium citrate dibasic (Sigma-Aldrich), 0.2 g/L of magnesium sulfate (Heptahydrate, Loba Chemie), 0.05 g/L of manganese sulfate (monohydrate, Loba Chemie), and 2 g/L of potassium phosphate dibasic (anhydrous, Loba Chemie). Distilled water was used for the preparation of the fermentation broth. A preparation of 500 mL sterile fermentation broth was made.

The fermentation was carried out in a glass flask of 1 L (GL 45, Duran®) during 31.5 h. An incubator (Binder™, ±0.1 K) was used to keep a constant temperature of 37 ºC along fermentation. Samples of 3 mL were taken each 2 hours in order to measure cell growth (by dry cell weight method), lactic acid and glucose (by HPLC). The pH (inoLab™, ±0.01) within the fermentation broth was measured during each sampling. For HPLC (ELITE LaChrom) analysis, an ORH-801 column (Chrom Tech) with a solution of 0.01 N H_2SO_4 (Merck, assay 95-97%) was used for the mobile phase, and a RI detector at 45 °C.

5.2.4 Results and discussion

Liquid membrane in Taylor flow for LA removal

In the sensitivity analysis of the OVMTC in the range of variables described in Table 1, the parameters of the OVMTC used for Eq. (3) were 1.6889, 0.5091, 0.1221, 2.7945, -0.0485, -1.3924 and, 0.0272 (α_1 to α_7). The calculation of the dimensionless numbers was carried out as shown in a previous work [4].

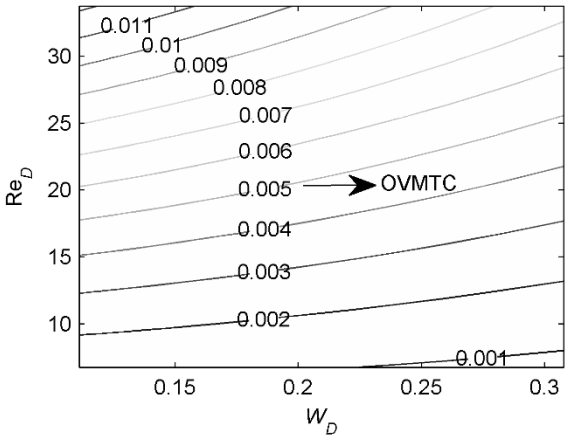

Figure 3. Effect of Reynolds and relative velocity on overall volumetric mass transfer coefficient (OVMTC, 1/s) at a channel length of 348.8 cm, an inner diameter of the channel of 2.5 mm, a slug length of 4 cm and an injected volume of the donor phase of 0.3436 cm^3.

For Taylor flow, an increase of the droplet velocity increases the mass transfer through the interfaces between the droplets (donor and receiving) and the continuous phase (membrane phase), because the intensity of the internal circulations (within the slug and droplet) and the renewal velocity of the liquid film (around droplets) also increase as the droplet velocity rises [22,23]. Internal mixing provides homogenization of the solute concentration within the droplets and within the slug [24–28].

If the liquid film around the droplets is continuously renewed, a high gradient of solute on the interface droplet/membrane phase can be achieved. Therefore, the OVMTC is enhanced with the increase of the droplet velocity. Hence, the OVMTC increases as Re_D rises (Figure 3).

Droplets in Taylor flow in liquid-liquid systems flow faster than the continuous phase due to the slip effect [29] provided by the liquid film that surrounds the droplets [30]. The relative velocity (W_D) becomes low as the velocity of the continuous phase is near the droplet velocity. The faster the continuous phase velocity, the higher the intensity of the internal circulations on the slug. Therefore, at low relative velocities, high values of the OVMTC are obtained (Figure 3).

The effects of slug length and the injected volume of donor droplets on the OVMTC are low (lower than 9.5%) compared with effects provided by the velocity of the donor droplets and the membrane phase (Re_D and W_D), where even the OVMTC can increase one order of magnitude (for instance, from 0.0008 to 0.0122 1/s, which is an increment higher than 90% on the OVMTC).

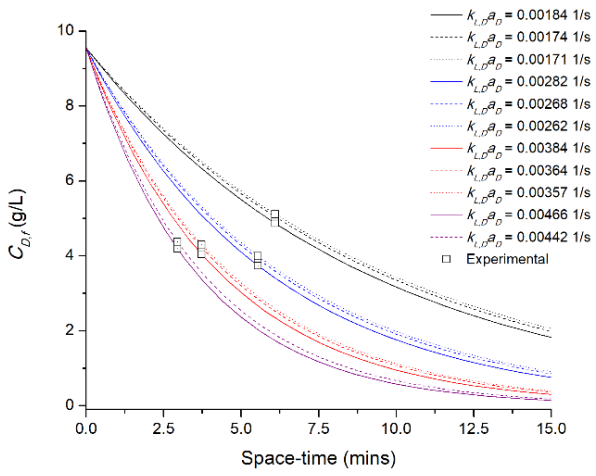

Figure 4. Experimental and calculated (by Eq (2)) final concentration of the donor droplets at the experimental values of the OVMTC at several values of the space-time with an initial LA concentration of 9.57 g/L in donor droplets.

The space-time is a variable that directly impacts on the amount of solute transported through the LMTF system. The space-time depends on the velocity of droplets and the channel length. The channel length was 348.8 cm for all reported experiments [4]. Therefore, the experimental values of the space-time only depend on the donor droplet velocity, which directly affects the OVMTC. The experimental space-times varied from 2.91 to 6.10 min (Figure 4). It is expected that a higher OVMTC corresponds to a lower required length of the channel that donor droplets should pass in

order to achieve the maximum LA transport in the LMTF (Figure 4). Furthermore, when the droplet has a low LA concentration, the effect of the space-time has a lower impact on the final LA concentration than when the donor droplets has a high LA concentration. If there is a high LA concentration in donor droplets, the driving force between donor droplets and the membrane phase is high and thus low space-times are required. For the highest OVMTC achieved in the sensitivity analysis (0.0122 1/s), 7 min is a sufficient space-time to achieve the highest LA transfer from donor droplets.

Batch lactic acid fermentation

The fitted kinetic model is in agreement with the experimental data of the batch fermentation (Figure 5), achieving coefficients of the determination (r^2) of 0.9973, 0.9956 and 0.9253 for glucose, lactic acid (LA) and biomass, respectively. From the fitted kinetic parameters (Table 2), it can be observed that the inhibitory LA concentration for production of LA and for cell growth is 13.94 and 14.49 g/L, respectively. In batch fermentation, when the LA concentration within the fermentation broth is achieving the inhibitory LA concentration, the glucose consumption and biomass production tends to cease. These occur in the pH range from 3.5 to 4.5.

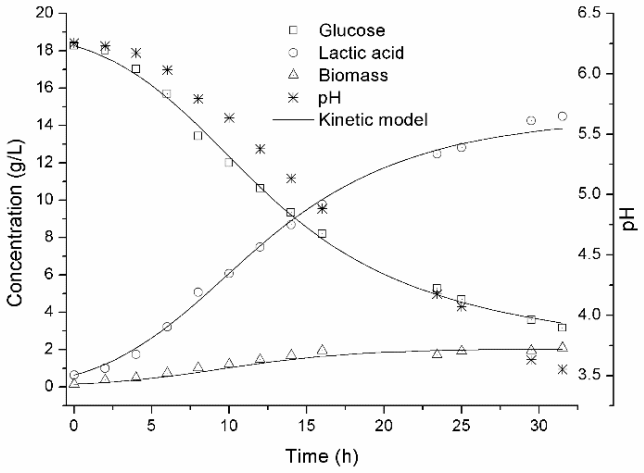

Figure 5. Experimental data (symbols) and fitted kinetic model (continuous lines) of the batch lactic acid fermentation by *Lactobacillus casei* ATCC 393 at 37 ºC during 31.5 h.

The batch lactic acid fermentation (Figure 5) shows that after 31.5 h of fermentation, glucose is still available to convert to LA. However, by the inhibitory effect of the LA concentration within the fermenter, the lactic acid bacteria cease the LA and biomass production at 25 h.

Table 2. Fitted kinetic parameters for the LA fermentation by *Lactobacillus casei* ATCC 393 at 37 ºC.

A	3.0061 g LA / g biomass
B	0.8994 g LA / g biomass·h
K_S	18.2720 g/L
μ_{max}	0.5215 1/h
P_{max}	13.938 g/L
P'_{max}	14.489 g/L
$Y_{P/S}$	1.8337 g LA / g glucose
$Y_{X/S}$	0.3227 g biomass / g glucose
m	0.0431 g glucose / h·g biomass

Integration of the LMTF with the lactic acid fermentation

The hybrid system was experimentally tested elsewhere [14] where all details can be shown. The fermentation process was modeled using Eqs. (4)-(7) for the times in batch, and Eqs. (15)-(17) and Eqs. (22)-(23) for the time of the hybrid process. The OVMTC was calculated by Eq. (3) from the experimental operating conditions, giving a value of 0.0033 1/s. The model developed in this work is in agreement with the experimental results (Figure 6) and the differences are analyzed below. The model shows that the LA removal is slightly higher than experimental because LA removal in the experiment was not strictly continuous. For the injection of the donor phase in the LMTF, a syringe pump was used, which requires periods of times to reload the syringe with the filtered fermentation broth. In the model, during the hybrid process, there is a continuous LA removal.

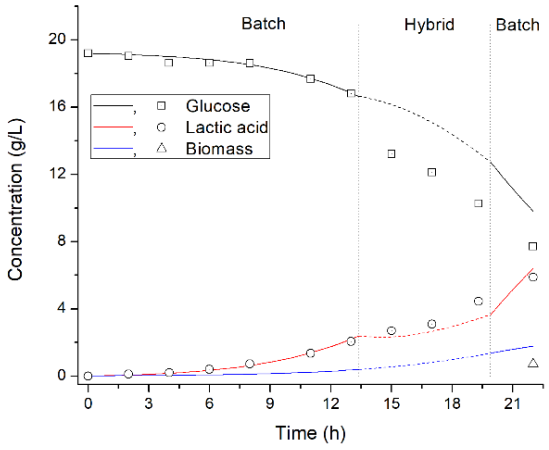

Figure 6. Experimental (symbols) and modeled (lines) batch-hybrid process during 22 h by using *Lactobacillus casei* ATCC 393.

The experimental data show that glucose is consumed faster in the hybrid period than in the non-hybrid period. However, this effect is not shown by the hybrid simulation. When the membrane phase is in indirect contact with the *Lactobacillus casei* ATTC 393, due a toxic effect the glucose consumption is promoted instead of the LA and biomass production [6,14]. This toxic effect is not included in the model explaining the differences in glucose concentration. In addition, the final biomass production is higher in the model than in the experiment due to the aforementioned molecular toxic effect.

The effect of the integration of the LMTF with one or several channels to the batch LA fermentation (of 50 mL) on the concentration profiles within fermenter was modeled by using Eqs. (15)-(17) and (22b)-(23b). For the LMTF the highest OVMTC (0.0122 1/s) achieved from the sensitivity analysis was used and the evaluation of the space-time (with a suitable space-time of 7 min) by Eq. (2), which are achieving for the operating conditions shown in Table 3. The hybrid system was modeled until full glucose consumption with LA removal through the LMTF with one channel from the beginning of the fermentation.

Table 3. Conditions which maximize the value LA removal through the LMTF by the assessment of the OVMTC and the space-time.

Variable	Value
L_{slug}	4 cm
V_D	0.3435 cm^3
Q_D	0.4545 cm^3/s
W_D	0.1111
Re_D	33.7491
Ca_D	0.0136

It can be observed that after 24.5 h of fermentation for the hybrid system (HS) there was a total glucose consumption (Figure 7a) achieving productivities of 0.5275 and 3.2853 g/(L·h), for batch and hybrid systems, respectively. The final biomass concentration was 1.9740 g/L for batch fermentation, while for the hybrid system it was 4.6786 g/L (Figure 7a). In the hybrid system, the rate of glucose consumption was practically constant as a function of fermentation time, which leads to a reduced fermentation time as compared to the experimental batch fermentation.

Additionally, within the fermenter, the LA concentration was kept below 4 g/L and the pH between 5.9 and 6.2 using only one channel of LMTF (Figure 7b). The LA concentration is kept low within the fermenter; the pH does not reach acidic conditions which are harmful to the lactic acid bacteria.

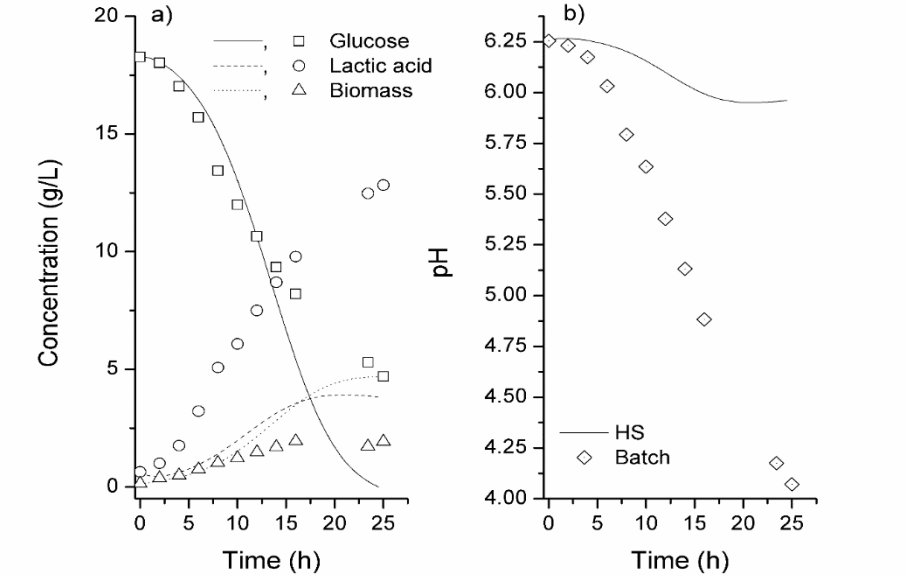

Figure 7. a) Profile of concentrations within the fermenter for the hybrid system (lines) at the best conditions of the LMTF, compared to experimental batch fermentation (symbols). b) Comparison of the pH within fermenter in hybrid (line) and batch (symbols) fermentations.

At acidic conditions, there is a high concentration of the undissociated LA, which is cytoplasmic membrane soluble, and as a consequence of its presence within the bacteria, the cellular functions are disabled [31]. This produces a failure in the proton motive forces of the cell [32]. Since the acidic condition within the fermenter was avoided by LA removal by LMTF, the lactic acid bacteria are not product inhibited, which allows for a normal glucose consumption to convert it in lactic acid, and keep the cell growth. Thus, the LA removal from the fermenter promotes glucose consumption, LA production, and cell growth.

The rate of LA removal in the hybrid system with one channel (Figure 8) was low from 0 to 5 h, because at this period of time, the driving-force for LA removal in the LMTF was low (Figure 7a). For times between 5 h and 20 h, the LA removal rate increases monotonically according to the increase of LA concentration within the fermenter and thus, the driving-force for LA removal. Hence, the rate of LA removal is proportional to the driving-force, but how close it is to the rate of LA production will be limited by the number of channels used in the LMTF system.

In spite of the similar rates of LA removal and LA production by using a LMTF with one channel (Figure 8), one more channel can be added to the LMTF to increase the rate of LA removal, reducing the fermentation time and increasing the productivity. However, the maximum rate of LA removal

that can be achieved is restricted by the rate of LA production, no matter if more channels of the LMTF are added. At this condition the LA concentration within the fermenter will be approximately constant. Hence, the main effect of increasing the number of channels of the LMTF for this volume of fermentation is on LA productivity and on LA concentration within the fermenter.

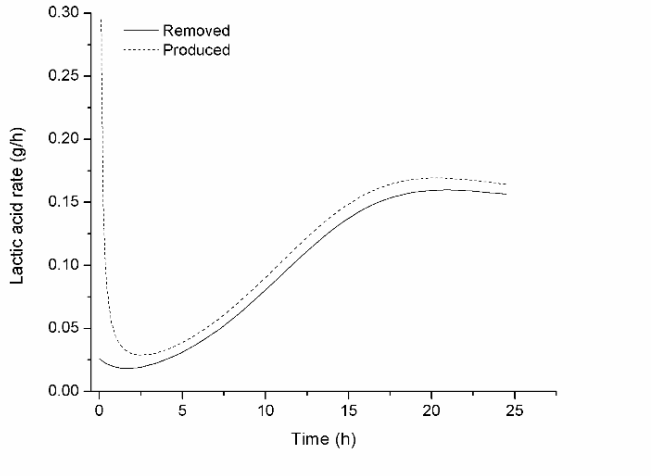

Figure 8. LA rates (removed and produced) of the hybrid system of the LMTF at the better conditions for the LMTF with one channel.

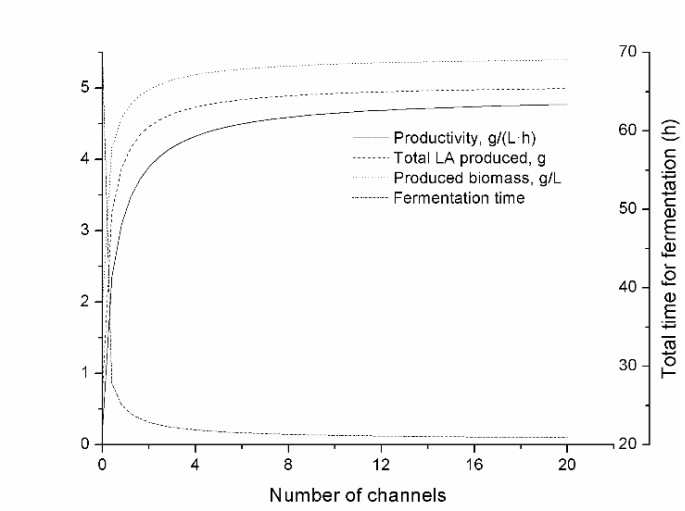

Figure 9. Effect of the number of channels on productivity (continuous line), the total mass of LA achieved (dash line), final biomass concentration (dot line) and required time for fully consumption of glucose (short dash-dot line) of the hybrid system for a batch fermentation of 50 mL.

Figure 9 shows that increasing the number of channels from 0 (conventional batch fermentation) to 4 reduces the fermentation time for total glucose consumption from 70 to 21.9 h, and the productivity increases from 0.2063 to 4.32 g/(L·h). For a number of channels higher than 4, there are no important changes on productivity, final mass of LA achieved, final concentration of biomass or the fermentation time. From these results, it can be observed that for every 50 mL of this fermentation broth, 4 channels are required in the LMTF using the best operating conditions of the LMTF based on OVMTC.

The removal of LA from the fermenter through the LMTF can also be useful to control the pH within the fermenter. It can be observed that the final value of the pH within the fermenter can be tuned by both varying the operating conditions and by the number of channels used in the LMTF (Figure 10). The LA fermentation has an optimal pH to the production of LA and biomass [33–35], therefore, the operating conditions and the number of channels of the LMTF, can be set according to required pH of the LA bacteria.

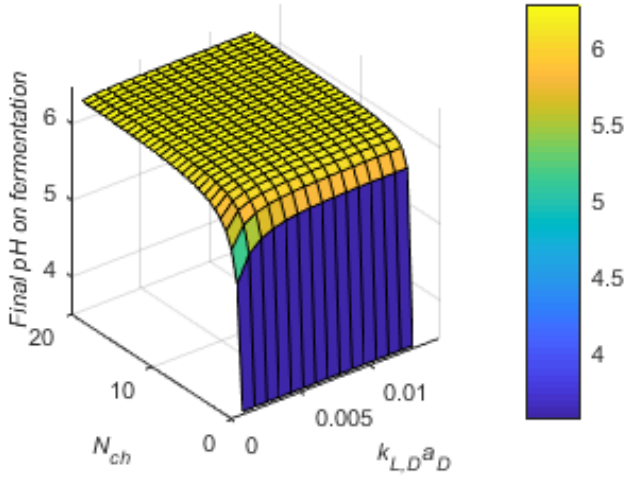

Figure 10. Effect of the OVMTC and number of channels of the LMTF on the final pH within the fermenter of 50 mL of fermentation broth.

In order to increase productivity, and the achieved values of the final biomass and LA concentrations, other schemes of the fermentation can be explored, for instance, a fed-batch fermentation integrated to a LMTF for LA removal. Although the LAB *Lactobacillus casei* ATCC 393 is inhibited at low LA concentrations it was possible to reduce the end-product inhibition opening the window to explore a continuous fermentation integrated with the LMTF. For this continuous fermentation, usually high biomass and substrate concentrations are used, in order to achieve high rates of consumption and production in the fermentation. However, some LAB does not tolerate high

substrate concentrations. An interesting advantage to explore by LA removal of a continuous or semi-continuous fermentation is that even when working at low substrate concentrations it is possible to achieve high consumption and production rates if the substrate is fed in different periods of time or in continuous and the LA is continuously removed by the LMTF. An economic study can be made in order to study the impact of applying these schemes or similar ones on the total cost of the process. For this cost analysis is important to include the toxic effect of the membrane phase on the lactic acid bacteria.

5.2.5 Conclusions

A model to describe a hybrid system of lactic acid removal by a LMTF from a fermentation broth was developed using a previous model of a LMTF and a kinetic model of a LA fermentation, which was fitted to experimental data of a batch fermentation. The model turns out to be in agreement to experimental data of the hybrid system and differences are due to toxicity effects that the model does not take into account. Using this model for the hybrid system, a range of operating conditions is recommended to increase the performance for LA production.

A high OVMTC of the LMTF for LA removal can be achieved at low relative velocities, high Reynolds, low injected volumes of donor phase and low slug lengths. However, the velocity of droplets and the membrane phase, which are involved in the relative velocity, are the main factors on the OVMTC.

By modeling the hybrid proposed system, it is possible to achieve low lactic acid concentration within the fermenter avoiding the acidic condition that negatively affects the lactic acid bacteria. Therefore, an enhancement of the productivity and the produced biomass is achieved. It was observed that at the modeling conditions, for each 50 mL of the fermentation broth 4 channels of the LMTF are required operating with the highest OVMTC of the LMTF. Additionally, the LMTF also can be used as a control system for the pH within the fermenter by tuning the operating conditions and the number of channels of the LMTF, in order to keep the pH at an optimum value within the fermenter according to the bacteria or strain used.

The fermentation time until total glucose consumption for a hybrid LMTF – fermentation system is reduced 3.19 times, while the LA productivity of the batch fermentation is increased 20.9 times as compared to a conventional batch fermentation.

NOTATION

A	Growth associate constant rate (g lactic acid/g biomass)
a	Specific surface area (m^2/m^3)
B	Non-growth associate constant rate (g lactic acid/(g biomass·h))
C	Concentration of solute (g/L)
Ca	Capillary number
d	Inner diameter of the channel (m)
F	Lactic acid mass flow (g/h)
J	Flux of solute (g/(L·s))
K_S	Monod constant (g/L)
k_La	Overall volumetric mass transfer coefficient (1/s)
L_{ch}	Channel length (m)
m	Coefficient of maintenance (g glucose/(g biomass·h))
N_{ch}	Number of channels in the LMTF
n	Number of experimental data
P	Lactic acid (product) concentration (g/L)
P_{max}	Lactic acid concentration above which bacteria do not grow (g/L)
P'_{max}	Lactic acid concentration above which bacteria cease lactic acid production (g/L)
Q	Volumetric flow rate (m^3/s)
Re	Reynolds number
S	Glucose (substrate) concentration (g/L)
t	Time (s)
U	Droplet velocity (m/s)
V_L	Fermenter volume (m^3)
W	Relative velocity
X	Biomass concentration (g/L)
$Y_{X/S}$	Biomass yield on the utilized substrate (g biomass/g glucose)
$Y_{P/S}$	Product yield on the utilized substrate (g lactic acid/g glucose)
α	Fitted parameter for calculation of the mass transfer coefficient
τ	Space-time (s)
μ	Specific growth rate (1/h)

Subscripts and superscripts

calc	Calculated data
D	Donor phase
eq	In equilibrium
exp	Experimental data
in	Input stream
inj	Injection
max	maximum
out	Output stream
slug	Slug
T	Total

5.2.6 References

[1] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications, 3rd ed., Elsevier, Amsterdan, 2003.

[2] V.S. Kislik, Liquid Membranes Principles & Applications in Chemical Separation & Wastewater Treatment, 1st ed., Elsevier B.V., Amsterdan, 2010.

[3] A.D. Pérez, J. Fontalvo, A new concept of liquid membranes in Taylor flow: performance for lactic acid removal, Chem. Eng. Process. - Process Intensif. (2019).

[4] A.D. Pérez, B. Van der Bruggen, J. Fontalvo, Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation, Chem. Eng. Process. - Process Intensif. 134 (2018) 20–27.

[5] J.A. Moulijn, A. Stankiewicz, J. Grievink, A. Górak, Process intensification and process systems engineering: A friendly symbiosis, Comput. Chem. Eng. 32 (2008) 3–11.

[6] A.D. Pérez, V.M. Gómez, S. Rodríguez-Barona, J. Fontalvo, Liquid-liquid Equilibrium and Molecular Toxicity of Active and Inert diluents of the Organic Mixture Tri-iso-octylamine/Dodecanol/Dodecane as Potential Membrane Phase for Lactic Acid Removal, J. Chem. Eng. Data. submited (2019).

[7] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using Lactobacillus casei ATCC 393, Dyna. 85 (2018) 360–366. doi:10.15446/dyna.v85n207.72374.

[8] A. Komesu, M.R. Wolf Maciel, R. Maciel Filho, Separation and Purification Technologies for Lactic Acid – A Brief Review, BioResources. 12 (2017) 6885–6901. doi:10.15376/biores.12.3.6885-6901.

[9] J. Vijayakumar, R. Aravindand, T. Viruthagiri, Recent Trends in the Production, Purification and Application of Lactic Acid, Chem. Biochem. Eng. Q. 22 (2008) 245–264. https://hrcak.srce.hr/24811.

[10] C. Miller, A. Fosmer, B. Rush, T. McMullin, D. Beacom, P. Suominen, Industrial Production of Lactic Acid, in: Ref. Modul. Life Sci., Elsevier, 2017: pp. 179–188. doi:10.1016/B978-0-12-809633-8.09142-1.

[11] M. Singhvi, T. Zendo, K. Sonomoto, Free lactic acid production under acidic conditions by

lactic acid bacteria strains: challenges and future prospects, Appl. Microbiol. Biotechnol. 102 (2018) 5911–5924. doi:10.1007/s00253-018-9092-4.

[12] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Ind. Eng. Chem. Res. 43 (2004) 5969–5982. doi:10.1021/ie049963n.

[13] N. Tik, E. Bayraktar, Ül. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, J. Chem. Technol. Biotechnol. 76 (2001) 764–768.

[14] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Integration of a liquid membrane in Taylor flow regime with a fermentation by Lactobacillus casei ATCC 393 for in-situ lactic acid removal, Chem. Eng. Process. - Process Intensif. submited (2019).

[15] J. Fontalvo, A.D. Pérez, Membrana Líquida y proceso para realizarlo, Rad. 15-131023, n.d.

[16] Y. Okubo, T. Maki, N. Aoki, T. Hong Khoo, Y. Ohmukai, K. Mae, Liquid-liquid extraction for efficient synthesis and separation by utilizing micro spaces, Chem. Eng. Sci. 63 (2008) 4070–4077. doi:10.1016/j.ces.2008.05.017.

[17] M. Sattari-Najafabadi, M.N. Nasr Esfahany, Intensification of liquid-liquid mass transfer in a circular microchannel in the presence of sodium dodecyl sulfate, Chem. Eng. Process. Process Intensif. 117 (2017) 9–17.

[18] D. Liu, K. Wang, Y. Wang, Y. Wang, G. Luo, A simple online phase separator for the microfluidic mass transfer studies, Chem. Eng. J. 325 (2017) 342–349.

[19] M. Sattari-Najafabadi, M. Nasr Esfahany, Z. Wu, B. Sundén, Hydrodynamics and mass transfer in liquid-liquid non-circular microchannels: Comparison of two aspect ratios and three junction structures, Chem. Eng. J. 322 (2017) 328–338.

[20] Susanti, J.G.M. Winkelman, B. Schuur, H.J. Heeres, J. Yue, Lactic Acid Extraction and Mass Transfer Characteristics in Slug Flow Capillary Microreactors, Ind. Eng. Chem. Res. 55 (2016) 4691–4702.

[21] M. Monteagudo, Lourdes, J. Rincón, J. Fuertes, Kinetics of Lactic Acid Fermentation by Lactobacillus delbrueckii Grown on Beet Molasses, J. Chem. Technol. Biotechnol. 68 (1997) 271–276.

[22] M.N. Kashid, D.W. Agar, S. Turek, CFD modelling of mass transfer with and without

chemical reaction in the liquid-liquid slug flow microreactor, Chem. Eng. Sci. 62 (2007) 5102–5109.

[23] N. Aoki, S. Tanigawa, K. Mae, A new index for precise design and advanced operation of mass transfer in slug flow, Chem. Eng. J. 167 (2011) 651–656.

[24] R. Gupta, S.S.Y. Leung, R. Manica, D.F. Fletcher, B.S. Haynes, Hydrodynamics of liquid–liquid Taylor flow in microchannels, Chem. Eng. Sci. 92 (2013) 180–189.

[25] H.L. Goldsmith, S.G. Mason, The flow suspensions through tubes. II. Single large bubbles, J. Coloid Sci. 18 (1963) 237–261.

[26] G.I. Taylor, Deposition of a viscous fluid on a plane surface, J. Fluid Mech. 9 (1961) 218.

[27] T.C. Thulasidas, M.A. Abraham, R.L. Cerro, Flow patterns in liquid slugs during bubble-train flow inside capillaries, Chem. Eng. Sci. 52 (1997) 2947–2962.

[28] T. Taha, Z.F. Cui, Hydrodynamics of slug flow inside capillaries, Chem. Eng. Sci. 59 (2004) 1181–1190.

[29] W.O. Smith, M.D. Crane, The jamin effect in cylindrical tubes, J. Am. Chem. Soc. 52 (1930) 1345–1349.

[30] F. Fairbrother, A.E. Stubbs, Studies in electro-endosmosis. Part VI. The "bubble-tube" method of measurement, J. Chem. Soc. (1935) 527.

[31] M. Othman, A.B. Ariff, L. Rios-Solis, M. Halim, Extractive Fermentation of Lactic Acid in Lactic Acid Bacteria Cultivation: A Review, Front. Microbiol. 8 (2017) 1–7. doi:10.3389/fmicb.2017.02285.

[32] L.M.D. Gonçalves, A. Ramos, J.S. Almeida, A.M.R.B. Xavier, M.J.T. Carrondo, Elucidation of the mechanism of lactic acid growth inhibition and production in batch cultures of Lactobacillus rhamnosus, Appl. Microbiol. Biotechnol. 48 (1997) 346–350. doi:10.1007/s002530051060.

[33] P. Pal, J. Sikder, S. Roy, L. Giorno, Process intensification in lactic acid production: A review of membrane based processes, Chem. Eng. Process. Process Intensif. 48 (2009) 1549–1559.

[34] C.N. Burgos-Rubio, M.R. Okos, P.C. Wankat, Kinetic study of the conversion of different substrates to lactic acid using Lactobacillus bulgaricus, Biotechnol. Prog. 16 (2000) 305–314.

doi:10.1021/bp000022p.

[35] A.D. Nandasana, S. Kumar, Kinetic modeling of lactic acid production from molasses using Enterococcus faecalis RKY1, Biochem. Eng. J. 38 (2008) 277–284. doi:10.1016/j.bej.2007.07.014.

6. Chapter 6: General conclusions and perspectives

6.1 Major findings

6.1.1 Liquid-liquid equilibria

The liquid-liquid equilibria (LLE) of organic mixtures of trioctylamine (TiOA) in dodecanol (at 37 vol% of TOA), tri-iso-octylamine (TiOA) in dodecanol (at 10 vol% of TiOA), TiOA in dodecane (at 10 vol% of TiOA) and TiOA in dodecanol/dodecane (at 10 vol% of TiOA and 40 vol% of dodecanol) with lactic acid (LA) aqueous solutions were experimentally measured and the LA distribution coefficient and LA chemical equilibrium constant were fitted through a proposed model. The tertiary amines react with LA to produce an acid-amine complex, and the diluents (dodecanol and dodecane) allow the solubilization of the free LA on the organic phase. On the other hand, dodecanol provides a solvation shell to the acid-amine complex to stabilize it. Therefore, the LA in the organic phase in equilibrium can be as free LA or as an acid-amine complex. However, most of the amount of LA in the organic phase in equilibrium is due to the chemical reaction which is in agreement with the results of the chemical equilibrium constant and distribution coefficients of the tested systems in this dissertation. For all tested systems, the distribution coefficient is around 0.01 and 0.26, while the chemical equilibrium is around 34 to 437 L/mol.

The organic mixture that provides a high LA concentration in the organic phase is TiOA in dodecanol. On the contrary, the organic mixture that provides the poorer LA concentration in the organic phase is TiOA in dodecane. Dodecanol is able to solvate the acid-amine complex, while dodecane does not provide solvation of the acid-amine complex. On the other hand, the mixtures TOA in dodecanol and TiOA in dodecanol/dodecane achieve similar values of the chemical equilibrium constant.

6.1.2 Molecular toxicity test combined with liquid-liquid equilibria assessments for a membrane phase selection

The tertiary amines in alcohols have shown enough capacity to receive the LA based on experimental results in this work and in the available scientific literature. However, generally, the solvents (for liquid-liquid extraction) and the membrane phases (for liquid membranes) with high capacity to LA removal are toxic to the lactic acid bacteria (LAB). However, the level of toxicity depends on the kind of microorganism and strain used. Hence, these solvents can be mixed with other organic compounds that reduce the toxic effect on the specific microorganism but also they can reduce the removal capacity. Liquid-liquid equilibrium is more critical for liquid extraction than for liquid membranes because with membranes the steps of extraction and solvent regeneration take place at the same time and thus the solvent bulk concentration is far from equilibrium.

In this thesis, the molecular toxicity of TOA, TiOA, Aliquat 336 (all of them are carriers), dodecane, dodecanol, and oleyl alcohol (all of them are diluents) on the LAB *Lactobacillus casei* ATCC 393 was experimentally measured (organic compounds with the potential to comprise membrane phase for LA removal). All carriers, tested are toxic on the LAB, following the next order of toxicity: Aliquat 336 > TOA > TiOA. For the diluents, the dodecanol is toxic, the oleyl alcohol is medium toxic and dodecane is non-toxic on the LAB. Also the molecular toxicity of TOA and TiOA at 10, 20 and 30 vol% in dodecane on the LAB was tested, showing that these tertiary amines at 10 vol% are non-toxic on the LAB, at 20 vol% they are medium toxic (being TiOA less toxic than TOA) and at 30 vol% the TiOA keeps medium toxic and the TiOA becomes toxic.

Then, the molecular toxicity and the LLE of TiOA at 10 vol% on dodecane and dodecanol at several concentrations of dodecanol (from 0 to 90 vol%) were experimentally tested. The mixture at 90 vol% of dodecanol (mixture TiOA/dodecanol) has higher values of distribution coefficient and chemical equilibrium constant than the mixture at 0 vol% of dodecanol (TiOA/dodecane), while the mixture TiOA/dodecanol is toxic and the mixture TiOA/dodecane is non-toxic on the LAB. Both, the molecular toxicity and the chemical equilibrium constant, for the dodecanol concentrations from 10 to 50 vol%, increases as dodecanol concentration rises. The mixture TiOA/dodecanol/dodecane at dodecanol proportions between 30 to 40 vol% shows a good compromise between a high value of the chemical equilibrium constant and a relatively low molecular toxicity.

An interesting finding of this study is the response of the LAB to the presence of the organic phases TiOA/dodecane and TiOA/dodecanol within the fermentation broth. The cell growth is promoted instead glucose consumption when the mixture TiOA/dodecane is within the fermentation broth,

while the glucose consumption is promoted instead cell growth when the organic mixture TiOA/dodecanol is within the fermentation broth. Even, for all mixtures TiOA/dodecanol/dodecane the last behavior persists, regardless of the presence of dodecane within the respective organic mixture.

6.1.3 Liquid membrane in Taylor flow

Removal of LA was used as a proof of concept of the liquid membrane in Taylor flow (LMTF). The LA removal level was experimentally measured through the degree of LA removal that is defined as the ratio between the amount of LA removed from donor phase and the total amount of LA acid that theoretically can be accepted in the receiving phase. According to experimental results, the degree of LA removal shows a dependency on the driving force of LA concentrations between donor droplets and membrane interface and the space-time of the phases within the channel. The maximum possible levels for the degree of LA removal are providing by low injection times of the dispersed phases and high droplet velocities in an optimal value of the slug length.

Additionally, the overall volumetric mass transfer coefficients (OVMTC) were calculated from the experimental results and a semi-empirical model was developed and fitted for the calculation of the OVMTC. The variables that most affect the value of the OVMTC are the donor droplet and membrane velocities, and the injection time, which are in agreement with the findings of the proof of concept of the LMTF.

The results show that the LMTF preserves the advantages of conventional emulsion liquid membranes while overcomes the stability problems of emulsion systems. Hence, the LMTF is a promising technology for industrial applications which can be integrated to other separation or reactive processes to enhance them by applying the intensification process philosophy.

6.1.4 Hybrid system of a LMTF integrated with a LA fermentation

The hybrid system was experimentally tested by integrating the LMTF for LA removal (using a membrane phase composed by TiOA/dodecanol/dodecane) with a LA fermentation with *Lactobacillus casei* ATCC 393. Through the hybrid system, it is possible to increase the biomass production and LA productivity by 12 and 41.8%, respectively, as compared to a conventional batch fermentation. However, the yields of LA to glucose and biomass to glucose are lower in the hybrid system as compared with the batch system. Due to molecular toxicity on the bacteria, glucose consumption is enhanced instead LA and biomass production.

A mathematical model that describes the hybrid system was developed using experimental data of conventional batch fermentation and the OVMTC model. The model for the hybrid system shows good agreement with experimental data of a hybrid system. However, there are some differences, especially on glucose concentration, due to the model does not take into account molecular toxicity. The model also includes the number of channels of a multi-channel LMTF. The impact of the multi-channel system on the LA productivity and biomass production are analyzed, showing that the end-product inhibition on the LA fermentation can be controlled by LA removal through the LMTF. Additionally, the LMTF is shown and proposed as a control system of the pH within the fermentation broth, which can optimize the LA production.

6.2 Perspectives

6.2.1 Scale up: Multi-channel system and phase separation in the LMTF

An interesting following step of this research is on developing of a new prototype of the LMTF including several channels in order to increase the capacity to process the donor phase through the LMTF. By a multi-channel LMTF prototype, the impact of the integration of this LMTF with any fermentation process will be higher, the productivity and yields may have a successful impact on the economy of fermentation processes. On the other hand, it encourages the study of continuous hybrid processes by integration of a LMTF with multi-channels with several kinds of fermentations.

The developing of a new prototype of the LMTF, requires the design of an automatic system for phase separation (donor from the membrane and receiving from membrane) at the outlet of the channel. It can be carried out by applying the interaction of hydrophobic or hydrophilic surfaces which can interact with the phases of the LMTF in order to separate each other. Currently, a master-thesis in our research group (Applications of New Technology Research Group, ANTGR) that explores the spatial location of the channels, the angle of contact between the phases and the interaction between the surfaces of channel walls with the phases was carried. It explored both experimental and CFD (Computational Fluid Dynamics) results for the involved phases of the current LMTF for LA removal. The results are promising, as regards it is possible to separate these phases by the interaction between the surface of the channel walls and the involved phases (donor, receiving and membrane). The phase separation applying the aforementioned concept of the affinity of the phases with the surface of the walls also has been studied for other researchers showing a promising method for phase separation in two-phase systems.

Other alternative for phase separation is to use a timer for the injected donor and receiving phases. A timer can be integrated to solenoid valves and Y-divisors (or T-divisor), in order to drive the train of donor droplets with the membrane phase for one side of the divisor, and the train of receiving droplets with the membrane phase for the other side of the divisor. This kind of system has to be design for modules of hundreds or thousands of channels of the LMTF prototype.

6.2.2 Modeling of the LMTF by CFD

Modeling of the hydrodynamics and mass transport by CFD is an important complementary study to carry out for a deep understanding of the involved phenomena in solute transport by the LMTF system. In our research group a master-thesis on this field is currently carrying out. It involves modeling of the hydrodynamics and the mass transport through the droplets of the LMTF for LA removal by using the same phases than this thesis. A wide range of operating conditions can be explored achieving detailed and microscopic results that can be compared with the current experimental results.

6.2.3 Supported liquid membrane in multiphase flow

The concept of the LMTF can be extended to the conventional supported liquid membrane (SLM). The SLM is classified as a perstraction method without phase dispersion. However, the SLM can become in a perstraction method with phase dispersion by using tubular supports in a double-tube system in Taylor flow. The tubular porous support has to be located within of another tube. The porous of the support has to be filled with the membrane phase by the conventional techniques for that. For the inner section of the support, the membrane phase flows as a continuous phase while the donor phase flows as donor droplets. At the outside of the inner section, the donor phase can be separated from membrane phase by the technique of the affinity of the liquid with the wall surfaces or by a decanter (Figure 1). For the annular section (section between the tube walls and the external surface of the support) the membrane phase that is separated from the donor phase flows as continuous phase in the annuli in counter-flow (for higher driving force of solute removal) while the receiving phase can travel as annuli Taylor droplets or any another kind of dispersed phase of two-phase flows regime, such as, dropply/bubbly, stratified or churn flows. At the outside of the annular section, both phases (receiving from membrane) can be separated by the same technique used for the inner section, and the membrane phase must be fed into the inner section in order to stay recirculating continuously the membrane phase in both section of the system. In this process, called supported liquid membrane in multiphase flow (SLMMPF) the solute is transported from the donor droplets to

the membrane phase within the inner section. Then, a portion of the solute within the membrane phase within the inner section is transported through the filled porous of the support to the annuli. Subsequently, both the portion of the solute transported and not transported through the filled porous now are transported from the membrane phase within the annuli to the receiving phase into the annular section.

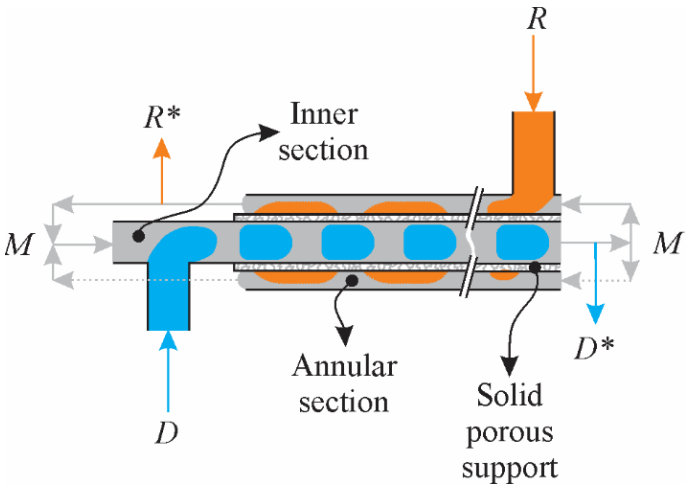

Figure 1. Scheme of a SLMMPF with Taylor droplets both in the inner section and in the annular section. *D*: Donor phase rich of solute. *D**: Donor phase poor in solute. *R*: Fresh receiving phase. *R**: Receiving phase rich in solute. *M*: Membrane phase.

The advantages of this proposed liquid membrane on its predecessor, the LMTF, are the two-phase flow formation and phase separation. In SLMMPF donor/membrane and receiving/membrane phases are flowing in two spatially separate sections, which provides of flexibility on the operation, because the formation of the donor droplets does not affect the formation for receiving droplets (or another multiphasic flow), and the collision and subsequent coalescence not can occur as in the LMTF can happen. On the other hand, both two-phase flows (donor/membrane and receiving/membrane) are continuously flowing for each section in the SLMMPF, while in the LMTF just one of the two-phase flows can occur or be produced at the same time. However, the solute transport rates depend on the kind of two-phase flow that will be used on any section of this system. Perhaps, the highest solute transport rates in the SLMMPF can be achieved by using Taylor flow in both sections of the SLMMPF, and it can be near to the solute transport rates of the LMTF. Additionally, this configuration provides a solution for the common stability problem of the conventional SLM.

Also, in the SLMMPF, the donor droplets can flow by the annuli while the receiving droplets by the inner section. The location for the dispersed phases will depend on the mass transfer rates among the

phases. In theory, the phase (donor or receiving) which provides of the lower transfer rate (taking into account the kind of two-phase flow used) must be located in the inner section in order to produce Taylor droplets and take advantage of the features of mass transport of this kind of two-phase flow. The absorption process (gas-liquid), such as the CO_2 recovery in a donor phase, also can be explored both the SLMMPF and the LMTF. There are several configurations to explore in this new proposed technology.

6.2.4 Possible industrial applications

The receiving phase and the membrane phase synthetized in for this work can be used for removal of other organic acids, such as citric, acetic, malic and butyric, among others. Also, on another fermentation process such as ethanol or ABE fermentation. For both fermentative processes, liquid-liquid extraction has been studied for the specific metabolite removal. Therefore, there are available membrane and receiving phases for it.

Another interesting field for the LMTF by using tri-iso-octylamine as the carrier within the membrane phase is the removal of some heavy metals from effluents, such as cadmium, chromium, cobalt, and lithium, where the conventional liquid membranes already are involved.

The window of applications for the LMTF process is wide. In theory, every single liquid-liquid extraction process or liquid membrane process can become in a LMTF process. Then, there are several fields to explore by using this promising membrane technology. For instance, applications in process that involve equilibrium chemical reactions (in the liquid phase) in order to remove one of the products and drive the reaction in the desired direction (by Le Chatelier's principle), or for enzymatic reactions which require the removal of the product. One example is the esterification reactions, where the ester production can be promoted by water or the ester removal (any product of the reaction). The challenge of these applications is in to find the suitable membrane and receiving phases for the specific solute removal.

List of Scientific Contributions

Articles published in peer-reviewed academic journals

- Alan D. Pérez, Sneyder Rodríguez-Barona, Javier Fontalvo, Liquid-liquid equilibria for trioctylamine/1-dodecanol/lactic acid/water system at 306.1, 310.1, and 316.1 K: Experimental data and prediction, J Chem. Eng. Data 61 (2016) 2269−2276.
- Alan D. Pérez, Bart Van der Bruggen, Javier Fontalvo, Study of overall mass transfer coefficients in a liquid membrane in Taylor flow regime: Calculation and correlation, Chem. Eng. Process. - Process Intensif 134 (2018) 20–27.
- Alan D. Pérez, Sneyder Rodríguez-Barona, Javier Fontalvo, Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using *Lactobacillus casei* ATCC 393, DYNA, 85(207), pp. 360-366, Octubre - Diciembre, 2018.
- Alan D. Pérez, Sneyder Rodríguez-Barona, Javier Fontalvo, Liquid−liquid equilibria of lactic acid/water solutions in tri-iso-octylamine/dodecane/1-dodecanol at 306.1, 310.1, and 316.1 K. Experimental data and prediction, J. Chem. Eng. Data, 2019, 64 (2), 603–610
- A.D. Pérez, J. Fontalvo, A new concept of liquid membranes in Taylor flow: performance for lactic acid removal, Chem. Eng. Process. - Process Intensif 139 (2019) 95–102
- A.D. Pérez, V.M. Gómez, S. Rodríguez-Barona, J. Fontalvo, Liquid-liquid equilibrium and molecular toxicity of active and inert diluents of the organic mixture tri-iso-octylamine/dodecanol/dodecane as potential membrane phase for lactic acid removal, J. Chem. Eng. Data. Article ASAP.
- A.D. Pérez, J. Fontalvo, Integration of a liquid membrane in Taylor flow regime with a fermentation by *Lactobacillus casei* ATCC 393 for in-situ lactic acid removal, Chem. Eng. Process. - Process Intensif. 140 (2019) 85–90.

Articles submitted in peer-reviewed academic journals

- Alan D. Pérez, Bart Van der Bruggen, Javier Fontalvo, Modeling of a liquid membrane in Taylor flow integrated with lactic acid fermentation, Chem. Eng. Process. - Process Intensif. *submited*.

Contributions to scientific conferences

- 10th European Congress of Chemical Engineering, Nice - France, September 27th to October 1st, 2015. A liquid membrane process using a Taylor flow regime – Oral presentation.
- Congreso Colombiano de Ingeniería Química y Profesiones Afines, Manizales - Colombia, 18 - 20 de octubre, 2017. Evaluación de un sistema híbrido de fermentación integrado a una membrana líquida en flujo de Taylor para la recuperación de ácido cítrico – Poster.
- Congreso Colombiano de Ingeniería Química y Profesiones Afines, Manizales - Colombia, 18 - 20 de octubre, 2017. Evaluación de la toxicidad de solventes orgánicos en bacterias probióticas - Poster.
- Congreso Colombiano de Ingeniería Química y Profesiones Afines, Manizales - Colombia, 18 - 20 de octubre, 2017. Separación de ácido láctico a través de una membrana líquida en flujo de Taylor – Oral presentation.
- Euromembrane, Valencia - Spain, 9 - 13 July, 2018. Liquid membrane in Taylor flow regime: Lactic acid removal. Poster.

International awards

- Best poster presentation award: Euromembrane 2018, Valencia – Spain, 9 – 13 July.

About the author

Alan Didier Pérez Ávila was born in Bogotá, Colombia in 1985. He earned his B.Sc. and M.Sc. in Chemical Engineering in the National University of Colombia, Manizales-Colombia. He is a member of the research group of application of new technologies since 2011. He was the technical laboratory coordinator in the laboratory of process intensification and hybrid systems (2014-2017). He was a teacher of kinetic and catalysis and teaching assistant in heat and transfer processes, both at National University of Colombia, Manizales-Colombia, in 2013 and 2014, respectively.

His research has focused on process intensification, working in the development and integration of membrane technology with fermentation processes. During his M.Sc. and Ph.D. studies, he has worked in developing a new kind of contact in liquid membranes by using the Taylor flow regime, under the supervision of Professor Javier Fontalvo. His Ph.D. internship was in the ProcESS division of KU Leuven, Belgium. Here, he worked under the supervision of Professor Bart Van der Bruggen as a guest researcher. During his internship, he works in developing a model for the liquid membrane in Taylor flow (LMTF) and for a hybrid system where the LMTF is involved. He is a member of the European Membrane Society since 2018.

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